Henry B. Morrison, J.

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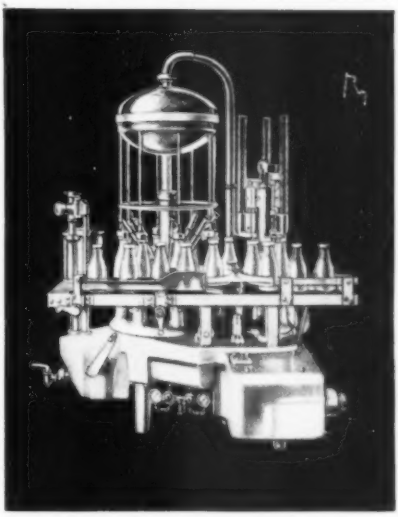

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American Dairy Science Association Announcements

AUTHOR ABSTRACTS

Arrangements are being made, at the request of Dr. J. R. Schramm, Editor of Biological Abstracts, to have authors prepare abstracts of their own articles as they appear in this Journal. Biological Abstracts will furnish forms and directions for preparing the abstracts and they will be received by the authors with the galley proof of their articles. The author abstracts will then be sent to the editor with the galley proof and the editor will forward them to Dr. Schramm. After considerable study Dr. Schramm found that best abstracts are secured promptly by this procedure and authors are urged to cooperate to assure the securing of good abstracts of their articles.

NEWS ITEMS

The executive committee at its meetings in Kentucky last June discussed various affairs of this Journal at considerable length. It was decided that the editor should endeavor to incorporate into this Journal the ideas expressed regarding news items.

Commencing with the next volume, this Journal will carry a section for news items to give personal as well as Association affairs of general interest. The editor believes that some person in each Dairy Department, U. S. Bureau of Dairy Industry, and other agencies with membership ought to be designated as correspondent to secure and send news items to the editor. Items of interest to our membership include brief statements concerning special honors bestowed upon members, promotions, new positions, deaths, special services, new buildings, special appropriations, etc. Activities of geographic divisions of the Association should be sent in by the officers of the divisions. Retiring officers of divisions ought to promptly notify the editor and secretary-treasurer of their successors.

The success of a news section is dependent primarily upon the cooperation of the membership and all are urged to send items to the editor. They will be published promptly without following the usual procedure for manuscripts. The news items should be sent now so that the section can start with volume 16. It will be combined with the present page of Association announcements as the closing page of each number of this Journal.

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THE HEAT COAGULATION OF MILK

II. The Influence of Various Added Salts Upon the Heat Stabilities of Milks of Different Concentrations

BYRON H. WEBB and GEORGE E. HOLM

Bureau of Dairy Industry, U. S. Department of Agriculture, Washington, D. C.

The colloidally dispersed calcium caseinate is the phase principally concerned in the coagulation of milk. It exists in a finely divided state (1) and is equilibrated, with respect to composition and charge, with the electrolytes in the serum in which it is dispersed. The particles are negatively charged and to a certain extent hydrated. To these properties they owe their colloidal stability.

On the basis of the theory of colloid stabilization and destabilization the addition of positively charged electrolytes to milk should tend to precipitate the calcium caseinate particles. It is significant, however, that addition of these electrolytes even in relatively large quantities at room temperatures does not cause precipitation. Addition of salts containing heavily charged negative ions should stabilize the calcium caseinate by further increasing the charge on each particle. But, contrary to the general rule for the stabilization of colloids, some milks, when judged by their resistance to coagulation with heat, are stabilized by addition of positively charged electrolytes while others are stabilized by electrolytes of the opposite character.

This has been shown in studies on heat coagulation by Sommer and Hart (2) and by Benton and Albery (3). Sommer and Hart have expressed the ratio of the amounts of positive ions (Ca and Mg) to that of negative ions (phosphates and citrates) as the "salt balance" (see our previous paper, p. 5) and have maintained that "a proper balance of these two classes of milk constituents will prevent the coagulation of the milk in the heat test; an excess of either one of these two causes the milk to coagulate." No definite meaning can be ascribed to the expression "proper balance" since the ratio is not a constant, and the type of electrolyte which will stabilize a milk as well as the amount is a matter of experimental determination.

The same authors have also concluded that "the salt balance, which was found to affect the coagulation of fresh whole milk at 136° C. also affects the heat coagulation of evaporated milk at the lower temperature used in commercial sterilization," and "The knowledge of salt balance gained by a

Received for publication December 10, 1931.

study of fresh milk samples at 136° C. has been successfully applied in preventing troublesome coagulation of evaporated milk."

However, results indicating that the additions of calcium do not produce analogous effects in an unevaporated milk and its evaporated product have also been noted by Sommer and Hart (2). It seems therefore evident that in dealing with the evaporated product wherein phase concentrations have been increased and salt equilibria altered, the reactions to heat are not analogous to those of the original milk. On the basis of the effect noted of various ions upon dilute and concentrated sols, the concentration of a milk should tend to decrease the relative precipitating effectiveness of the di- and trivalent, and increase the effectiveness of the monovalent ions. The effect of forewarming and concentration upon the salt constituents can not be surmised.

The results presented in our previous paper, as well as of those reported by Sommer and Hart and by Benton and Albery, indicated therefore that a more comprehensive and detailed study of the effect of different added electrolytes upon the heat stability of milks of different concentrations was necessary to aid in clarifying the problems.

COAGULATION WITH HEAT IN THE PRESENCE OF ADDED ELECTROLYTES

Preliminary to the work on the effects of electrolytes on coagulation by heat, the relationships of time and temperature of coagulation were determined in order that a temperature might be chosen which would properly reflect stability variations. The relationship of forewarming, a commercial practice, to the stability of samples of a milk of different concentrations was also established in order that the results obtained might be properly interpreted in the light of commercial practice.

In the following work the time of coagulation of the milks was obtained by heating them in sealed tubes immersed in a glycerine bath, at 120° C. unless another temperature has been given. Skimmed milks were used throughout, since it has previously been shown (4) that the presence of small percentages of fat in its natural state of dispersion does not change the heat stability relations involved.

Aqueous salt solutions of different concentrations were made up. The required amount of salt solution, not to exceed 1 cc., was pipetted into a test tube together with enough distilled water to give a total of 1 cc. of aqueous solution, when less than 1 cc. of salt solution was used. To this was added 5 cc. of the milk to be tested, making the total volume in the tube equal to 6 cc.

The Time-Temperature Relationship

The effect of various temperatures from 80° to 120° C. upon the time of coagulation of several milks of 9 per cent and 18 per cent solids-not-fat

concentration was determined in the manner described. The results, plotted in Fig. 1, show that as the temperature is increased the time of coagu-

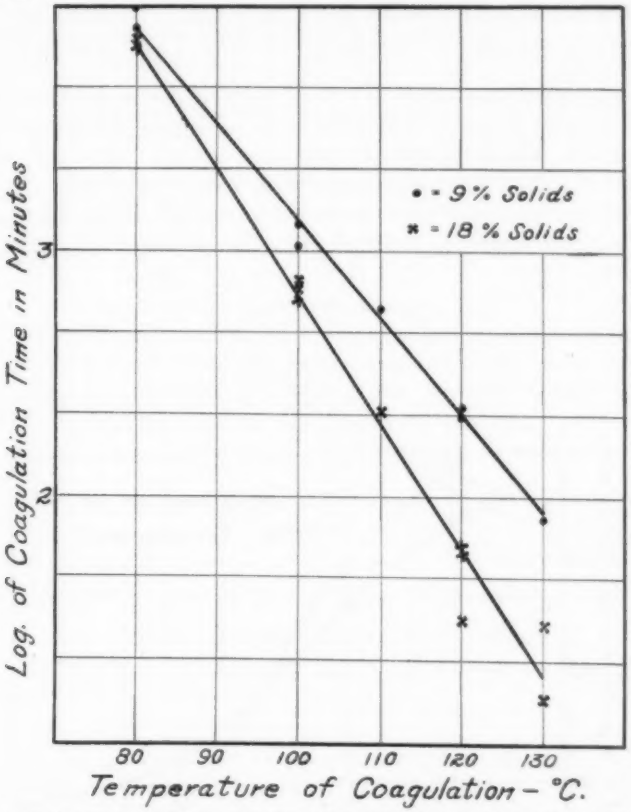

FIG. 1. THE RELATIONSHIP BETWEEN THE TEMPERATURE AND TIME OF COAGULATION OF SKIMMED MILK OF 9 AND 18 PER CENT SOLIDS CONTENT.

lation decreases logarithmically, although the rate of decrease in the case of milks of 18 per cent solids-not-fat is greater than in those of 9 per cent solids-not-fat concentration.

The milks used in the above experiments were of good quality and of approximately the same stability to heat. Milks of lower quality would yield results of a lower order of magnitude (4). In these latter cases the results obtained with the samples of 18 per cent solids-not-fat concentration at temperatures of 130° C. or above are somewhat high, due in part to the proportionately large value of the time necessary for the samples to come to the temperature at which they are to be maintained.

The data indicate also that, aside from the practical impossibility of using the lower temperatures, they do not yield results on milks of 9 per cent solids-not-fat concentration whose differences are directly comparable with those obtained on the same milks reduced to 18 per cent solids-not-fat concentration.

In experiments carried out at 115° C. or at lower temperatures, the time element involved is too great to make these conditions practical, especially in the case of skim milks of 9 per cent concentration. Too high temperatures, 130° C. and higher, give results with skim milks of 18 per cent concentration that may be in error as heretofore stated. Hence a temperature of 120° C. was chosen as optimum from the standpoint of experimental conditions.

Forewarming

The temperature of forewarming of a milk evaporation is known to influence the heat stability of its concentrated product. The effect of forewarming changes, however, with a change in the solids-not-fat content of the milk. The magnitude of this change is shown in figure 2. The data

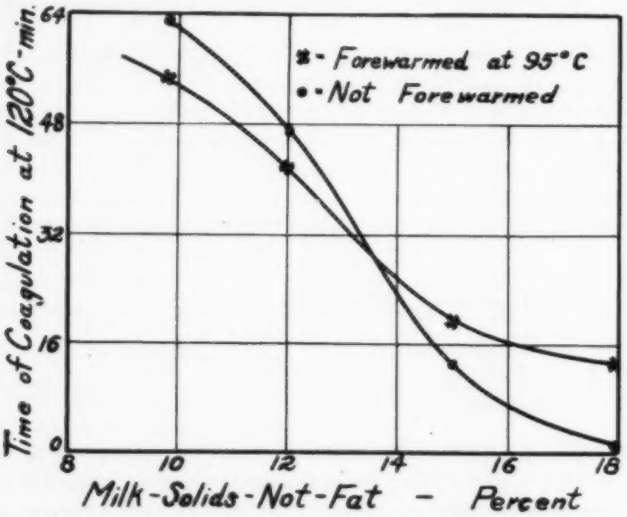

FIG. 2. THE EFFECT OF FOREWARMING UPON THE HEAT STABILITY OF A SKIMMED MILK REDUCED TO VARIOUS CONCENTRATIONS.

in this figure were obtained by forewarming part of a batch of milk to 95° C. for 10 minutes before evaporation. The control sample was not forewarmed. The two milks were then evaporated to the specified concentrations and their heat stabilities were determined. The results show that milk having a solids-not-fat content of 13.5 per cent is not changed in sta-

bility by forewarming, but in milk below this value forewarming is detrimental and at concentrations above 13.5 per cent stability is improved. This critical solids content was found to range from 10.5 to 14 per cent, depending upon the individual milk used.

A similar relationship is shown in figure 3. In this case the milk used was increased in heat stability by the addition of the proper amount of

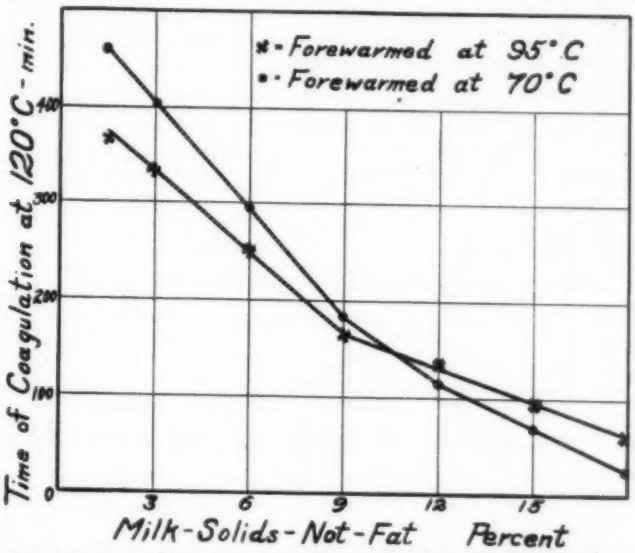

FIG. 3. THE MAXIMUM HEAT STABILITY WHICH WAS ATTAINED BY THE ADDITIONS OF $AlCl_3$ TO SKIMMED MILKS FOREWARMED TO 70° C. AND 95° C., RESPECTIVELY, AND REDUCED TO VARIOUS SOLIDS CONCENTRATION.

$AlCl_3$. Excessive amounts of the salt decreased stability. The figure shows the maximum stability which it was possible to attain in the milk by additions of $AlCl_3$. Different percentages of solids were used and each milk was forewarmed to 95° C. and to 70° C. The critical solids content where forewarming does not affect stability is here shown to be 10.5 per cent.

The effect of the addition of $CaCl_2$ upon the heat stability of a milk of 18 per cent solids which had been forewarmed to 95° C. and to 65° C. is shown in figure 4. This is a type of milk which is increased in stability by calcium additions and will be discussed later. It is interesting to note that the curves are similar but represent different degrees of stability, which is controlled by the temperature of forewarming.

Figures 3 and 4 show that there is a fundamental change of unknown nature which is brought about by forewarming. This change is not

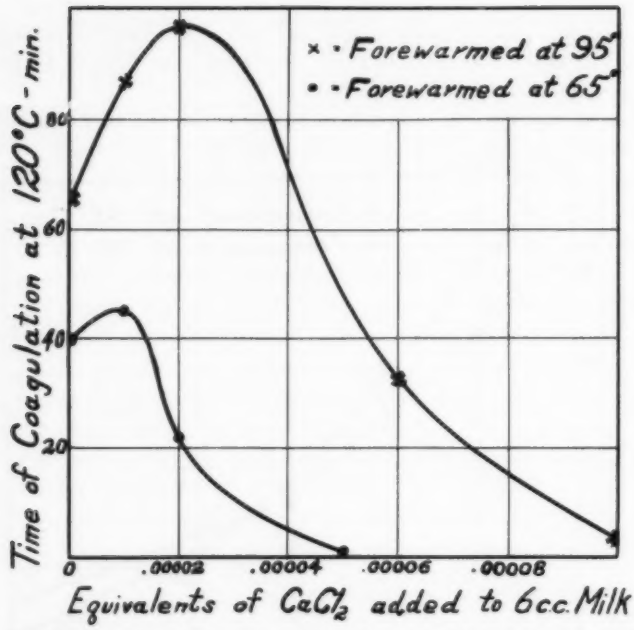

FIG. 4. THE EFFECT OF THE ADDITION OF $CaCl_2$ UPON THE HEAT STABILITY OF SKIMMED MILKS OF 18 PER CENT SOLIDS CONCENTRATION AND FOREWARMED TO 95° AND 65° C.

affected by additions of salts to the milk. The same relationship between milk forewarmed to 95° C. and 65° C., as that shown in figure 4, was obtained when $BaCl_2$, $AlCl_3$, $ThCl_4$, K_2HPO_4, or $K_2C_6H_7O_5$ was used in place of $CaCl_2$. It was found impossible to restore the stabilities obtained for milk forewarmed at 65° C. to the high stability resulting from 95° C. forewarming by the addition of any of the above salts. In no case was the higher temperature curve lowered to coincide with the 65° C. curve when equal quantities of the same salt were added to milks forewarmed to each of these temperatures, respectively.

The results indicate that similar conditions of forewarming must be practiced in all cases where comparisons are to be made and that the addition of electrolytes does not alter the relationship established by forewarming except in degree of stability. It appears that forewarming does not affect stability merely through changes in the equilibria of the soluble salts since the stability cannot be restored by the addition of electrolytes in those cases wherein it has been reduced. Forewarming seems therefore to cause an irreversible change which appears to be corollary to other changes

caused by heat. This change may be one of variations in the hydration of the calcium caseinate particle due to alteration in its composition.

Two Types of Milk

It has been shown by previous investigators (2) (3) (5) that two different types of milk exist with respect to the effect of the hydrogen ion concentration of the milk upon its heat stability. It has been demonstrated that the addition or bacterial development of acid in a milk may either increase or decrease its stability. The final effect depends upon securing in the milk the optimum pH for the particular sample in question.

Investigations on the effect of pH upon the heat stability of a milk have not previously included results which directly compared the fresh milk with its concentrated product. In figures 5, 6 and 7 are plotted data ob-

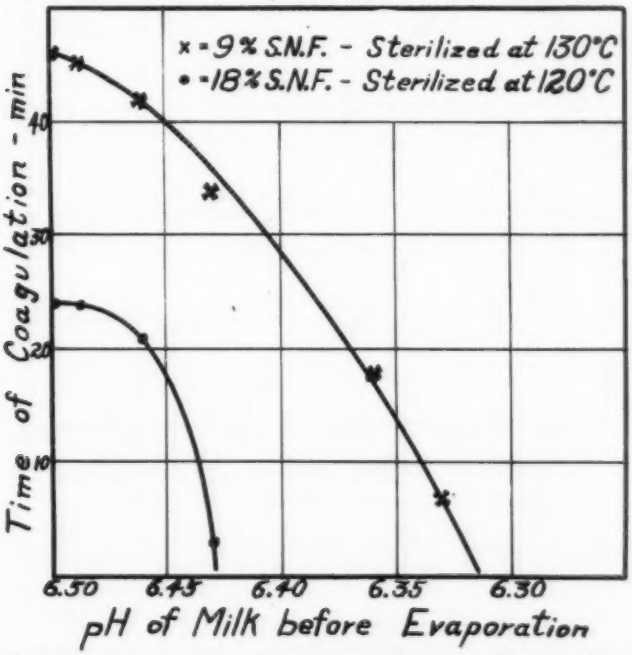

FIG. 5. THE EFFECT OF H^+ ION CONCENTRATION UPON THE HEAT STABILITY OF ONE TYPE OF FRESH MILK AND OF ITS CONCENTRATED PRODUCT. MILK FOREWARMED TO 95° C. BEFORE EVAPORATION. ACIDITY OBTAINED BY THE ADDITION OF LACTIC ACID TO THE RAW MILK.

tained by making such a comparison, using different types of milk. In figures 5 and 6 the increase in acidity was produced by the addition of lactic acid to and by the action of bacteria upon the fresh unconcentrated

skimmed milk, respectively. Half of each sample was then evaporated to 18 per cent solids and the heat stability of all samples determined. The data shown in figure 7 were obtained by the addition of HCl to fresh and concentrated samples of a single milk just before sterilization.

The milk upon which the values shown in figure 6 were obtained is typical of the majority of normal milks. An increase in acidity decreased the stability of both the fresh and concentrated samples. An increase in heat stability was brought about in both the fresh and evaporated milks shown in figure 6 by a small increase in acid. Milk of this type which is not al-

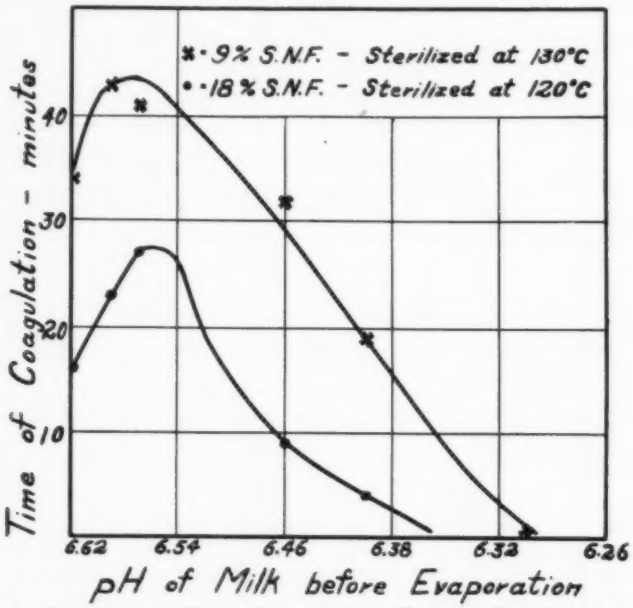

FIG. 6. THE EFFECT OF H^+ ION CONCENTRATION UPON THE HEAT STABILITY OF ONE TYPE OF FRESH MILK AND OF ITS CONCENTRATED PRODUCT. MILK FOREWARMED TO 95° C. BEFORE EVAPORATION. ACIDITY OBTAINED BY THE ACTION OF BACTERIA DURING NORMAL AGING OF MILK.

ready at its optimum pH to give maximum stability toward heat is frequently encountered. The results shown in figure 7 were obtained from a milk which was different from either of the two preceding types. Addition of HCl to the concentrated sample increased its stability to heat. Obviously in a milk of this type it would be impossible to apply to the concentrated product any results obtained with the initial milk as Sommer and Hart found could be done in their work. Concentration not only lowers stability but alters the effect of acids upon the subsequent stability of the

milk. Two types of milk exist, therefore, with respect to their stability to acids and the degree to which their normal concentrations are affected is not necessarily reflected in their concentrated forms.

Effect of Various Electrolytes Upon Heat Stability

The facts cited question the use of buffer salts to stabilize all milks toward heat. Furthermore, it is known that the hydrogen ion has a high degree of activity in affecting ζ-potential and stability changes of various colloidal dispersions, and the question naturally arises to what extent other ions can promote the same change. This question becomes more important

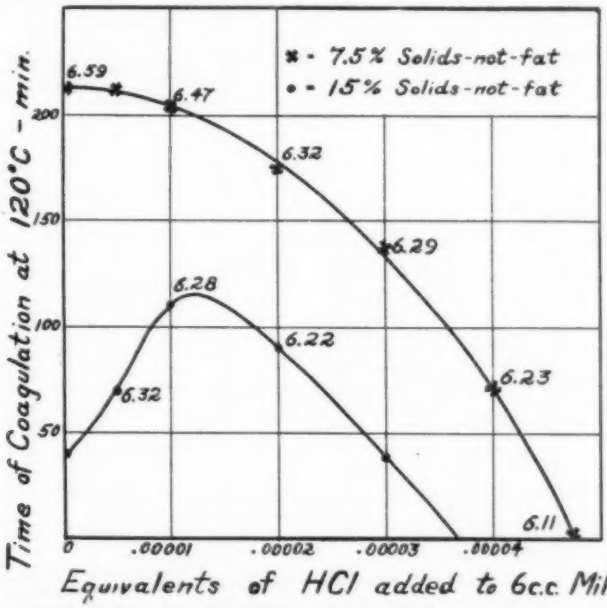

FIG. 7. THE EFFECT OF THE ADDITION OF HCl UPON THE HEAT STABILITY OF ONE TYPE OF FRESH MILK AND OF ITS CONCENTRATED PRODUCT. MILK FOREWARMED TO 95° C. BEFORE EVAPORATION. THE pH VALUES AFTER EACH ADDITION OF ACID ARE INSERTED IN THE FIGURE.

when it is considered that there is a great difference in the magnitude of the action of ions of different valences in various colloid sols.

The quantitative effect of mono-, di-, tri- and quadrivalent ions upon milks of various concentrations was first studied.

The effect of the addition of different electrolytes upon the heat stability of skim milks of different solids content is shown in figures 8 to 14. The salts used were KCl, HCl, $BaCl_2$, $CaCl_2$, $AlCl_3$, and $ThCl_4$. Complete

curves were obtained for each salt and for milks of several concentrations. The data extend from no additions of salt to addition of large enough quantities of salt to decrease the stability of the milk to a value of zero minutes at 120° C. The data, given in figures 8 to 14, were obtained on different milks, although each separate figure is plotted from data obtained with a single milk.

Figures 8 to 14 show also that a certain critical salt concentration existed beyond which rapid sensitization of the sol to heat occurred. The

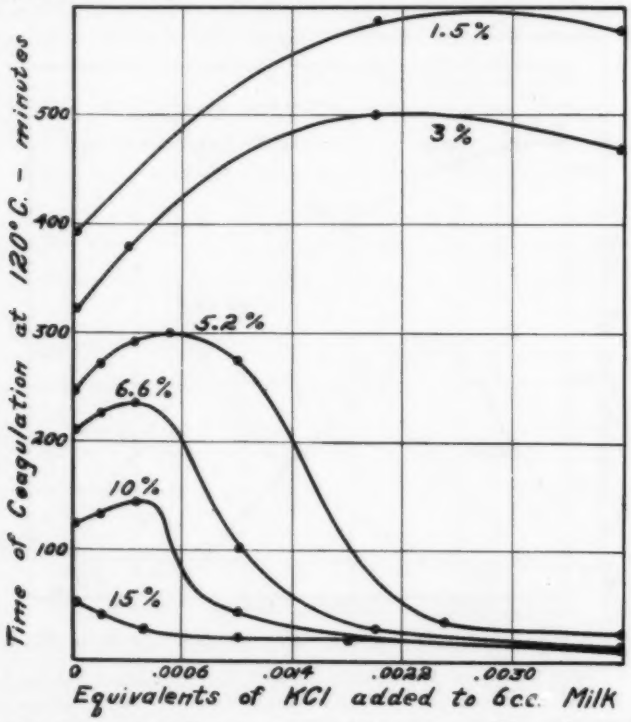

FIG. 8. THE EFFECT UPON HEAT STABILITY OF ADDITIONS OF KCl TO SKIMMED MILKS OF DIFFERENT SOLIDS CONCENTRATION

value of this critical salt concentration was dependent upon the solids content of the milk and upon the precipitating ion. As the percentage of milk solids in the sol decreased, the fall from maximum stability to immediate coagulation became more abrupt and less salt was required to bring about this sharp drop in stability. The abruptness of the drop when the critical salt concentration was reached appeared to be dependent upon the nature of the precipitating ion. Hydrogen, thorium, and aluminum caused rapid

changes in heat stability after passing the critical salt concentration while potassium, barium and calcium had a milder effect.

The foregoing results show that the milks of lower concentrations are the most sensitive to the effect of tri- and quadrivalent positive ions. The higher concentrations are more sensitive to variations in the amounts of the mono- and divalent positive ions, HCl and Ba and Ca salts being highly effective at all concentrations. The relationship is similar to that determined for As_2S_3 sols with K, Ba and Al ions (6, p. 449). It seems, there-

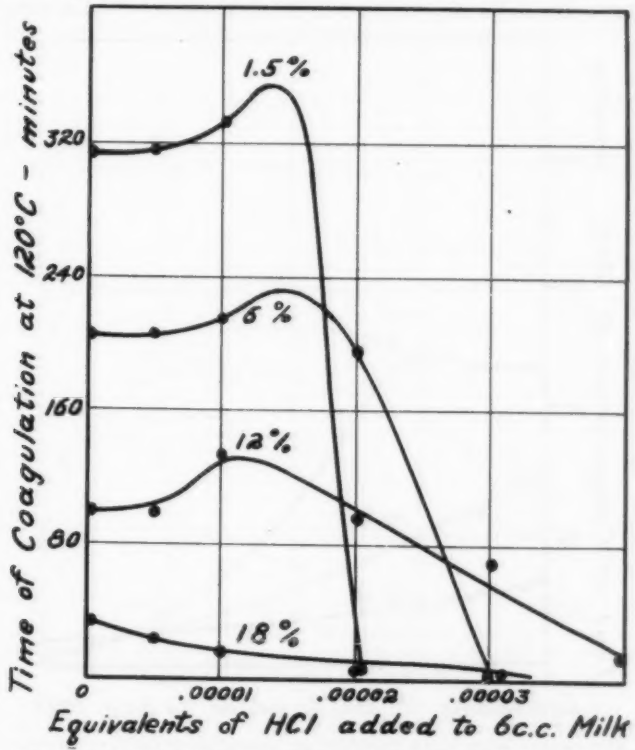

FIG. 9. THE EFFECT UPON HEAT STABILITY OF ADDITIONS OF HCl TO SKIMMED MILKS OF DIFFERENT SOLIDS CONCENTRATION.

fore, that the calcium caseinate suspensions are somewhat analogous to hydrophobic sols in their reactions toward electrolytes.

The effect of additions of different quantities of di-potassium acid phosphate and potassium citrate upon the heat stability of a fresh skimmed milk is shown in figure 14. Attempts were also made to adjust the pH of the milk to that of the original sample before the additions of salt had changed

it. Two curves, with and without pH adjustments, for both citrate and phosphate additions have been included in figure 14.

The effect of the phosphate and citrate ions upon the stability of fresh milk is not the result of a change in pH. The results given in figure 14 show that when the pH is not adjusted after the salt additions to that of the original milk, the stability is only a little greater than when the pH is lowered by HCl additions.

The abrupt decline in the citrate curves in figure 14 is noteworthy. The same effect, although less marked, would be shown in the phosphate curves

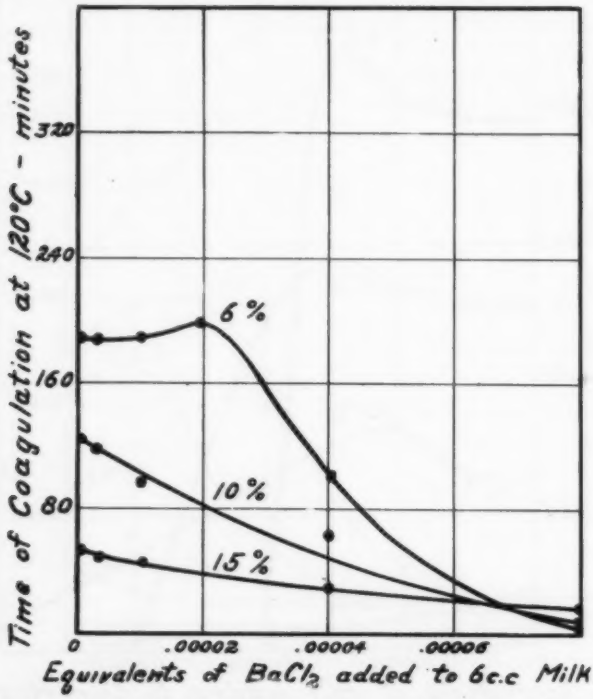

FIG. 10. THE EFFECT UPON HEAT STABILITY OF ADDITIONS OF $BaCl_2$ TO SKIMMED MILKS OF DIFFERENT SOLIDS CONCENTRATION.

if they were extended farther than is shown in the figure. This rapid decrease in heat stability of an unconcentrated milk with very small additions of salt probably indicates that the potential on the particle has reached its critical value. Stability has increased to a maximum at the point where a proper balance in particle charge has been attained. The delicate balance is completely upset by further salt additions.

The results in figure 14 show, especially in the case of citrate additions, that the range of maximum stability lies within comparatively narrow limits with respect to salt concentrations.

Sommer and Hart (2) found that citrates produced greater stability than phosphates for a given quantity of added salt. The data of figure 14 is in agreement with this finding. Potassium citrate produces a stronger and more powerful effect upon heat stability than does the phosphate. The maximum stability which can be attained by the addition of these negative ions is not, however, widely different.

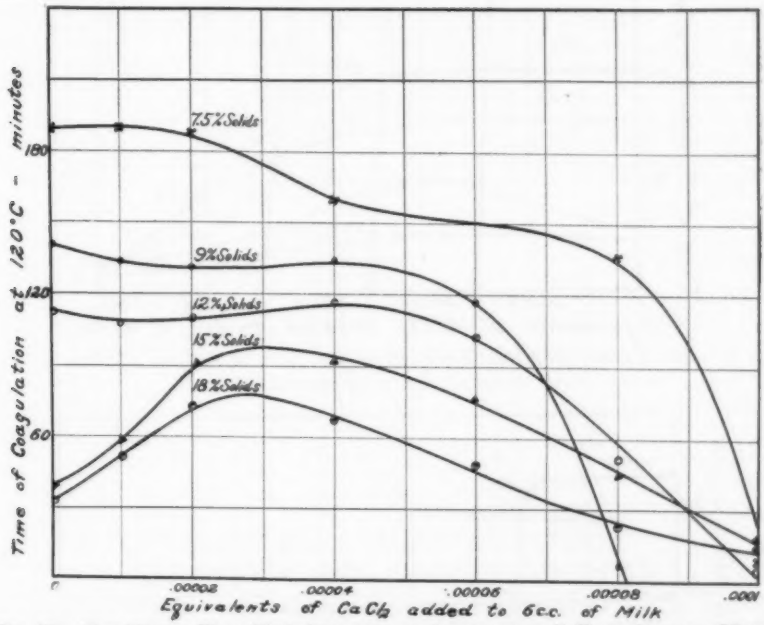

FIG. 11. THE EFFECT UPON HEAT STABILITY OF ADDITIONS OF $CaCl_2$ TO SKIMMED MILKS OF DIFFERENT SOLIDS CONCENTRATION.

It should be mentioned in passing that where no compensation was made for the increase in pH which occurred after large additions of citrate and phosphate to the milks, there was a marked tendency for the calcium caseinate to pass into solution. The first noticeable opalescence occurred when the amount of citrate exceeded 0.0003 and of phosphate 0.001 equivalents per 6 cc. of milk of 7.5 per cent solids after dilution. Reference to figure 14 will show that the curves were practically completed before this quantity of salt was used.

The work previously described has shown that with respect to the effect

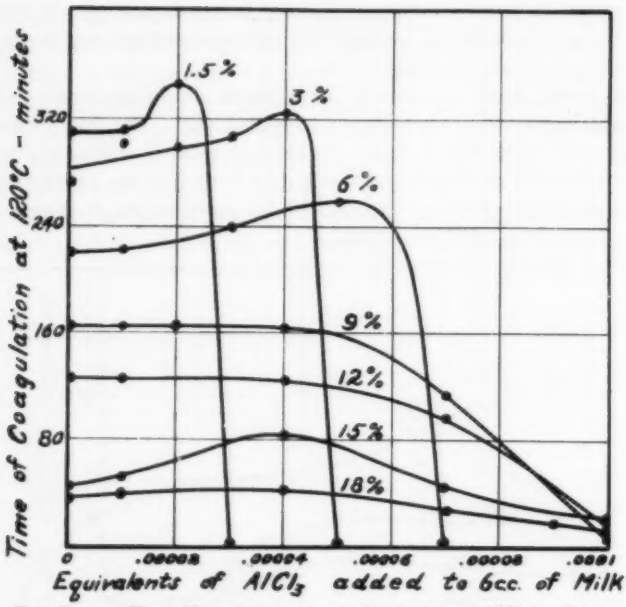

FIG. 12. THE EFFECT UPON HEAT STABILITY OF ADDITIONS OF $AlCl_3$ TO SKIMMED MILKS OF DIFFERENT SOLIDS CONCENTRATION.

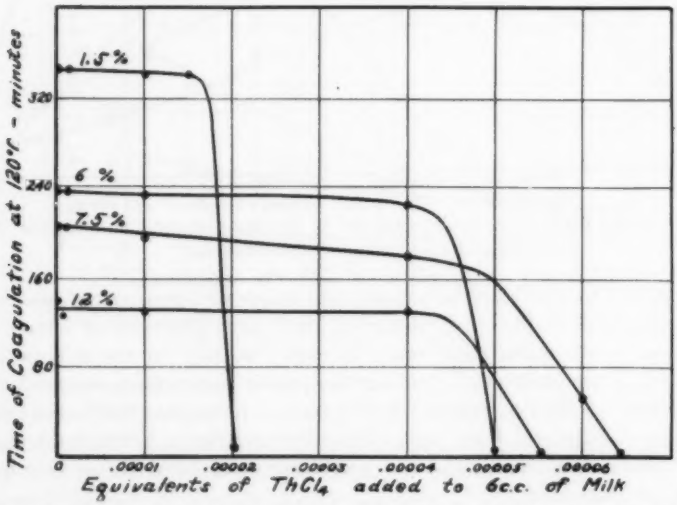

FIG. 13. THE EFFECT UPON HEAT STABILITY OF ADDITIONS TO $ThCl_4$ TO SKIMMED MILKS OF DIFFERENT SOLIDS CONCENTRATION.

of various positive and negative ions two types of milk exist. The magnitude of the effects produced has also been shown.

The effect of ions of different valence upon the two types of milks of high solids-not-fat concentrations is shown in figure 15. The milks used were mixed herd milks secured from the same herd on different days. All the samples were forewarmed to 95° C. for 10 minutes before evaporation. The curves which show stabilization by the different cations are plotted from data which were obtained from the same milk sample (milk of Jan.

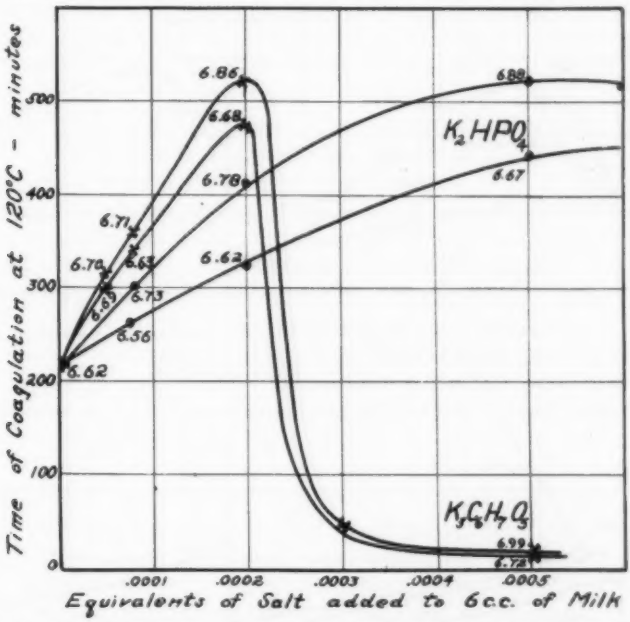

FIG. 14. THE EFFECT OF ADDITIONS OF POTASSIUM PHOSPHATE AND POTASSIUM CITRATE UPON HEAT STABILITY OF PLAIN SKIMMED MILK WHEN THE pH IS AND IS NOT READJUSTED BY ADDITIONS OF HCl AFTER THE SALT ADDITIONS. THE INSERTED FIGURES ARE THE pH VALUES. THE SOLIDS CONTENT OF THE MILKS AFTER ADDITION OF SOLUTIONS WAS 7.5 PER CENT.

7, 1931) concentrated to give a final milk solids content of 15 per cent. The lower group of curves in figure 15, in which notable stabilization was obtained with K_2HPO_4, are plotted from milks concentrated to yield a final solids content after dilution with salt solution of 18 per cent. These curves were obtained from milks secured on the following dates:

1. KCl $BaCl_2$ and $AlCl_3$—Aug. 5, 1930.
2. HCl —Sept. 9, 1930.
3. $CaCl_2$ K_2HPO_4 —Jan. 12, 1931.

Three separate milks are, therefore, concerned in the work shown in this part of the figure.

The fact that one set of curves is from milk of 15 per cent solids and the other, 18 per cent solids, does not lessen the value of the comparison between these two types of milk. The effect would be the same if both milks were standardized to either solids content, except that a small difference in coagulation time would result from the concentration differences.

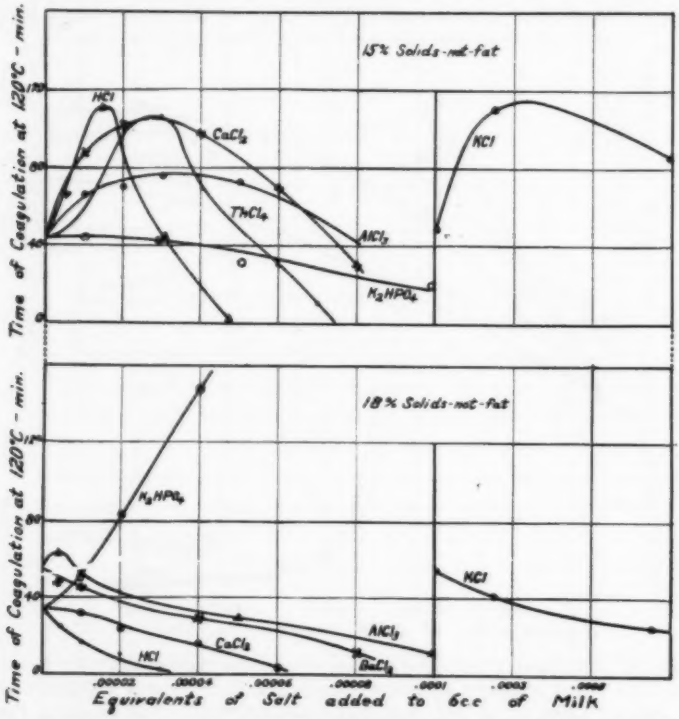

FIG. 15. THE EFFECT OF THE ADDITION OF VARYING AMOUNTS OF DIFFERENT ELECTROLYTES UPON THE HEAT STABILITY OF TWO DIFFERENT TYPES OF EVAPORATED MILK. ALL MILKS WERE FOREWARMED TO 95° C.

It has already been shown that the critical concentration range is 10.5 to 14 per cent solids, which is below that of each of these milks.

Figure 16 shows clearly the effect which different ions may exert upon the heat stability of an evaporated milk. The opposing effect of the calcium and phosphate ions upon stability as first shown by Sommer and Hart (7) is demonstrated and extends almost over the entire range of salt additions practically possible for each milk. It is interesting to note that where

the added ion increases stability, this additional heat resistance is very great, amounting in some cases to two or three times the original coagulation period of the milk.

Cations other than calcium can have the same effect as this ion, a fact which clearly demonstrates that the action of added calcium ions in changing the heat stability of a milk is not the result of the calcium itself but rather of the positive charges which the ion carries. It would, therefore, appear that in place of using Sommer and Hart's term of salt-balance, the necessary condition which must exist to secure maximum stability in a milk could better be described as a balance in particle charge. The effect of strongly positive cations is opposed to the action of heavy anions such as citrate or phosphate. Evidently there is a critical balance in the charge on the particle which is the criterion of maximum stability.

The work of Sommer and Hart (2) has shown that many unstable milks can be increased in stability by additions of calcium or of phosphate ions, respectively. It was believed by these workers that if a fresh milk was stabilized by either one of the above mentioned ions, the evaporated product of such a milk would also be stabilized in a similar manner. While this line of reasoning may apply to some milks, it is not typical in all cases as shown by the previous as well as the following experiments.

The effect of additions of $CaCl_2$ and of K_2HPO_4 upon the heat stability of the fresh and concentrated milks of four individual cows and of two composite herd milks are tabulated in table 1. The reaction of the fresh milks toward alcohol is also given.

In every case the addition of a calcium salt decreased the stability of these milks when they were of 7.5 per cent solids-not-fat concentration, while the addition of a phosphate had the opposite effect. This has been found to be generally true with very few exceptions.

However, when the milks were condensed to give a solids-not-fat concentration of 18 per cent, the addition of the calcium salt decreased the stability of the milks from Cows 271, 812 and 807, while the milk from Cow 280 and the herd milks were increased in stability. Addition of the phosphate increased the stability of the milks from Cows 812 and 807, and decreased that of the mixed herd milks.

Complete data upon the action of different electrolytes when added to unevaporated and evaporated milks of these types are plotted in figure 16.

The curves for the milks of each cow were all obtained from the same lot of milk from that cow. The concentrated milks were forewarmed to 95° C. for 10 minutes before evaporation. The directly opposite manner in which the fresh and concentrated milks from both cows react toward the added salts is very striking.

The fresh milk of Cow 280, which was alcohol negative, was increased in stability by the addition of K_2HPO_4, while both HCl and $CaCl_2$ rendered

TABLE 1

Effect of $CaCl_2$ and K_2HPO_4 upon the time of coagulation of fresh and concentrated skimmed milks from the milks of different cows. Concentrated milks were forewarmed to 95° C. before evaporation. Solids-not-fat percentages are those obtained after dilution with water or salt solution

QUANTITY OF SALT SOLUTION ADDED TO 6 CC. OF MILK	COW 271 ALCOHOL +		COW 812 ALCOHOL —		COW 807 ALCOHOL +		COW 280 ALCOHOL —		NO. 1 MIXED HERD MILK		NO. 2 MIXED HERD MILK	
	7.5% S.N.F.	15% S.N.F.	7.5% S.N.F.	15% S.N.F.	7.5% S.N.F.	18% S.N.F.	7.5% S.N.F.	18% S.N.F.	7.5% S.N.F.	15% S.N.F.	7.5% S.N.F.	15% S.N.F.
Equivalents	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*
No salt	105	14	301	214	127	34	218	23	212	44	243	41
$CaCl_2$												
.00001	91	11	263			32		31		88		
.00002	78	9	251	197	115	23	196	44	174	102	209	75
.00004	60	6	220	174	60	16	171	119	144	98	176	89
.00006	38	<5	211	144	0	<5		92		70		58
.00008	<5		203					66	118	30		
.0001									5			
K_2HPO_4												
.00001		14		214		50		12		46		
.00002	113	15		219	132	83		12		45	305	30
.00004	118	17		224	138		310	15	.285	36		
.00006	127	18	385	230		230		17			339	27
.0001						245		18	350	22		

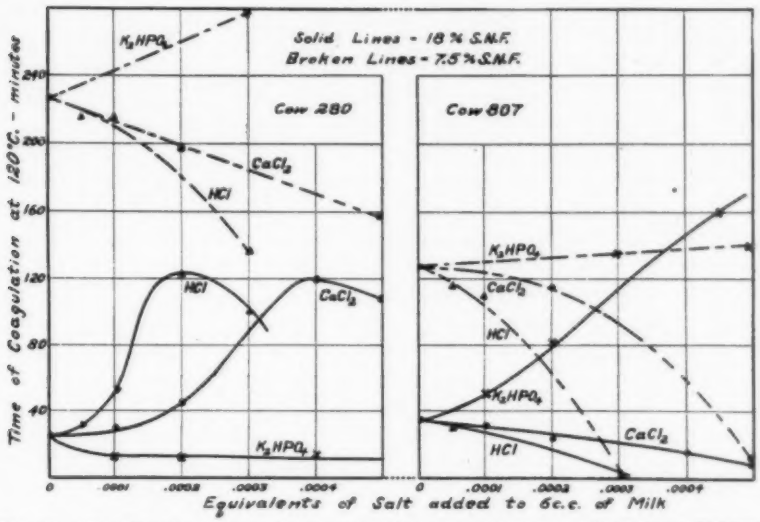

FIG. 16. THE EFFECT OF THE ADDITION OF VARYING AMOUNTS OF DIFFERENT ELECTROLYTES UPON THE HEAT STABILITY OF NORMAL AND EVAPORATED SAMPLES OF TWO DIFFERENT TYPES OF MILK.

it less stable. After this milk was concentrated the effect of these same ions was reversed, a very great increase in stability was produced by additions of $CaCl_2$ and HCl and a decrease by additions of K_2HPO_4.

The milk of Cow 807, which was alcohol positive, behaved in an exactly opposite manner to the milk of Cow 280, as is shown in figure 16.

Sommer and Binny (8) found that by increasing the magnesium or calcium content of a milk the alcohol test became positive, while additions of KCl, NaCl, citrates or phosphates did not cause a positive alcohol reaction. It seemed, therefore, possible that the two different types of milk used to obtain the data in figure 15 might be identified by their alcohol reactions. Accordingly, the milks of some thirty cows which were available for experimental purposes were tested to determine their reaction to alcohol. Unfortunately, Cow 807 was the only animal in this group that was producing alcohol positive milk. It was necessary, therefore, to postpone further work along this line until such a time as more alcohol positive milk might become available. However, the data on the milks of Cows 812 and 280, shown in table 2, both of which were alcohol negative, show that they reacted in different manners when calcium ions were added.

The data presented show quite conclusively that the stability of a milk to various ions is not always reflected in that of its evaporated product. They indicate also the probable reason for the possible use of phosphates

in different types of milk without disastrous results. In those cases wherein the addition of phosphate does not stabilize the evaporated product, the stability is not materially decreased. Consequently, the general use of a phosphate stabilizer might at one time be very beneficial while at other times it might produce no increase in stability, nor would it prove decidedly harmful.

With respect to the qualitative, as well as the quantitative, effect of various ions upon the stability of milks the data indicate that comparisons should be made only when comparable quantities of electrolytes, from the standpoint of their effectiveness, are used. For example, results obtained with the use of quantities of an electrolyte in a range wherein they are extremely effective cannot be compared with results obtained with quantities of an electrolyte that lie within the range of little effectiveness. Results obtained under such conditions are apt to lead to erroneous interpretations of the relative value of various electrolytes as stabilizing or destabilizing agents. For accurate interpretation of results it is, therefore, necessary to obtain data over a wide range of concentration of the electrolyte under consideration.

SUMMARY

The variations in the time of coagulation with variations in the temperatures used have been shown to be of a logarithmic nature. However, these variations in the time of coagulation are less with milk of 9 per cent solids-not-fat than they are when milk of 18 per cent solids-not-fat is used. The results indicate also that the use of temperatures of from 110° to 120° C. yield results of greatest practical value. Higher temperatures are apt to give results wherein the errors are large.

2. The data presented upon forewarming also indicate that the concentration of solids-not-fat is an important factor in varying the relative effects of this procedure. The results show that forewarming at high temperatures lowers the stability of those of higher solids-not-fat concentration. Hence, in any comparison of results obtained with milks of different solids-not-fat concentration similar treatment with respect to forewarming must be assured.

These results indicate also that the relationships established by forewarming are not altered by the addition of electrolytes except in degree of stability. It appears, therefore, that forewarming does not affect stability merely through changes in the equilibria of the soluble salts, as the stability cannot be restored by the addition of electrolytes in those cases wherein it has been reduced.

3. The results show conclusively that the heat stability of a milk of 18 per cent solids-not-fat concentration cannot be predicted from the heat sta-

bility of the original milk. They show further why the use of phosphates as a "neutralizer" has not led to serious trouble. In those cases wherein its use has not materially improved stability its use has not greatly reduced it.

4. The existence of two types of milk, one stabilized by the addition of positively charged electrolytes and the other stabilized by the addition of negatively charged electrolytes, has been confirmed. The effect of the addition of various electrolytes to these types of milk has been studied over a wide range of concentration and it has been shown that in the lower concentrations they are most sensitive to the effect of di- tri- and quadrivalent ions and the monovalent H-ion while in the higher concentrations the monovalent K-ion is also very effective. The relationships of these effects are similar to those found for As_2S_3 sols. Hence it again seems to have been shown that the calcium caseinate suspension in milk is analogous to hydrophobic sols in its reactions with electrolytes at higher temperatures. Increased stability has been shown to result from the addition of citrates and phosphates to a milk stabilized by negatively charged electrolytes. Finally, complete data have been presented upon the effect of various electrolytes upon the two types of milk at 9 and 18 per cent solids-not-fat concentrations.

In general, a study of the data presented shows clearly that in studies on coagulation careful consideration must be given to phase concentrations and that conclusions drawn from work on milk of one concentration are not applicable to other milks of widely different concentrations. Furthermore, at the lower concentrations of solids-not-fat various electrolytes exhibit critical concentrations or ranges wherein their effect is great. Hence, any work which does not include results over a range of concentration of electrolyte as well as range of concentration of the sol used is apt to lead to erroneous conclusions.

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THE INCORPORATION OF AIR DURING THE HOMOGENIZATION OF CREAM AND OF ICE CREAM MIXES

O. E. WILLIAMS AND ALAN LEIGHTON

Bureau of Dairy Industry, U. S. Department of Agriculture, Washington, D. C.

It is not generally known that during the homogenization of cream and ice cream mixes there may be an increase of volume (exclusive of surface foam) due to the incorporation of a small amount of air. This air incorporation is not necessarily permanent but may persist through the cooling process and until after the fluid is stored. Apparently the phenomenon is met with but occasionally and causes no difficulty except as it may cause considerable inconvenience in obtaining an accurate inventory of the stored cream. It is the belief of manufacturers that the condition is met with when defective packings of valves in the homogenizer permit air to be drawn into the machine and mixed with the fluid while processing. In an ice cream factory a number of years ago this volume increase of cream was estimated to be from four to five per cent of the original volume. The volume increase has been variously estimated to amount to as much as 15 per cent.

There seems to be no written record of the amounts of air that can be incorporated in cream or ice cream mixes during processing nor has any consideration been given to possible effects upon air incorporation of such factors as the temperature and pressure of homogenization. For that reason a series of experiments was undertaken to determine in a general way the conditions favorable or unfavorable to the incorporation of air in a fluid during homogenization and the amount of volume increase resulting under the conditions of the tests.

EXPERIMENTAL

The purpose of the tests was to determine the amount of air incorporated in creams and ice cream mixes during the homogenization process as compared with unprocessed control samples.[1] The factors considered for cream were homogenization pressure, homogenization temperature, the clogging of the homogenizer valves, and fat content. In the case of ice cream mixes the factors considered were fat content, homogenization temperature and pressure.

The amount of air incorporated is indicated by the increase in volume of the liquid under test. This volume is most easily measured by noting

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[1] A 90-gallon Progress Homogenizer with gravity feed through standard fittings was used in this experiment. The valves in this homogenizer are in good condition.

the change of density of the fluid during the course of the experiment as compared with its original density. The usual procedure was to homogenize the fluid, cool it on a surface tubular cooler, or if too viscous to flow readily, to cool it in a tank of ice water. Samples were then taken in weighed graduated 500 ml. flasks, brought to a uniform temperature, and the liquid level was adjusted to the mark and the flasks re-weighed.

The presence of foam made it very difficult to fill the flasks by pouring the cream into them, as foam would pour with the liquid cream. This difficulty was overcome by drawing the cream into the measuring flask from the bottom of the container.

The per cent change in volume of the liquid under experiment was calculated from the equation:

$$\text{Per cent change in volume} = \frac{\text{Density of control}}{\text{Density of treated material}} 100$$

An attempt was made to use a Westphal balance in determining the density but the high viscosity produced in some of the creams made it impossible to obtain accurate measurements by this method.

EXPERIMENTS

Three creams containing 20, 25, and 30 per cent fat, respectively, were each pasteurized at 145° F. for 30 minutes; then each was divided into five separate lots. The first lot (Control) was cooled without homogenization; the second lot was homogenized at 145° C. with 1000 pounds pressure; the third lot was homogenized at 145° F. with 3000 pounds pressure; the fourth lot was cooled to 128° F. and homogenized with 3000 pounds pressure; and the fifth lot was cooled to 110° F. and homogenized with 3000 pounds pressure.

The volume increases are given in table I.

These data show that neither changes in pressure nor small changes in temperature of homogenization are important factors in affecting the volume or density of homogenized cream containing 20 to 30 per cent fat. It is evident, however, that if extremely low temperatures are used the volume of the cream may be appreciably increased. There is a greater tendency for air incorporation in the higher fat creams but this tendency is not particularly marked and is apparently not always met with as evidenced by the next series of experiments.

The effect of faulty flow of cream into the homogenizer on air incorporation was investigated by closing one of the intake valves. In this way only two valves of the homogenizer were pumping cream normally while the other might possibly be pumping air into the cream. This caused the gauge hand of the homogenizer to be unsteady and jump from 1000 to 2500

TABLE I

Per cent volume increase of creams containing 20, 25 and 30 per cent fat

PER CENT FAT	LOT NO.	HOMOGENIZATION		EXPERIMENTS			
		Pressure	Temperature	No. 1 Volume increase	No. 2 Volume increase	No. 3 Volume increase	Average increase
		Pounds	°F.	*Per cent*	*Per cent*	*Per cent*	*Per cent*
20	1	.....	145	.....	.....	.....	.....
	2	1000	145	.00	.06	.04	.03
	3	3000	145	.38	.24	.18	.26
	4	3000	128	.46	.45	1.04	.66
	5	3000	110	.98	.69	3.08	1.59
25	1	.....	145	.....	.....	.....	.....
	2	1000	145	.02	.04	.00	.02
	3	3000	145	1.00	.04	.08	.38
	4	3000	128	.09	.80	1.24	.72
	5	3000	110	1.98	1.86	2.98	2.28
30	1	.....	145	.....	.....	.....	.....
	2	1000	145	.24	.26	1.32	.61
	3	3000	145	1.39	.32	1.52	1.08
	4	3000	128	1.92	.20	.88	1.00
	5	3000	110	4.25	1.50	1.40	2.38

pounds with the uneven action of the three valves. The data are recorded in table II.

TABLE II

Effect of closing one homogenizer intake valve on air incorporation in cream

Per cent fat in cream	VOLUME INCREASE OF CREAM OF DIFFERENT PER CENT FAT CONCENTRATION		
	20	25	30
	Per cent	*Per cent*	*Per cent*
Homogenized 145°F. (2500 lbs.)	3.15	2.66	1.28
Homogenized 110°F. (2500 lbs.)	11.16	5.18	3.44

More air was incorporated in these creams than in those of the preceding series of tests when the homogenizer valve was not blocked. However the amount decreased as fat increased. It is evident therefore that defects in the homogenizer action may give abnormal air inflations in cream.

No report has ever reached the authors of difficulties in manufacture due to air incorporation in ice cream mixes during homogenization. However, in order to determine whether or not homogenization did tend to incorporate air in ice cream mixes tests were made on two different types of mixes, *i.e.*, high and low fat content mixes. The data is given in table III.

TABLE III

Per cent air incorporated in homogenized ice cream mixes of 16 and 12 per cent fat content

LOT NO.	HOMOGENIZATION		ICE CREAM MIX	
	Pressure	Temperature	16 per cent fat	12 per cent fat
	Pounds	°*F.*	*Per cent*	*Per cent*
1	Control		0.00	0.00
2	1000	145	.26	.34
3	3000	145	.26	.14
4	3000	128	.88	.04
5	3000	110	1.06	.06

Here the air incorporation is not markedly different in amount between the ice creams of high and medium fat content except in cases where the temperature was lowered in homogenization of the 16 per cent mix. The lower temperatures, however, did not seem to affect the inflation of the lower fat mix.

DISCUSSION AND SUMMARY

In this series of experiments conducted to determine the amount of air that might be incorporated in creams and ice cream mixes during homogenization the maximum increase in volume that was attained under normal conditions was 4.25 per cent. When one homogenizer valve was clogged the maximum increase in volume amounted to 11.16 per cent. These values were not attained under normal conditions unless the homogenization temperature was unusually low. At the usual homogenization temperatures, or at those near pasteurization temperature, no significant increases in volume were obtained in either the cream or ice cream mixes.

SUBSTANCES ADSORBED ON THE FAT GLOBULES IN CREAM AND THEIR RELATION TO CHURNING. I. THE CHURNABILITY OF ARTIFICIAL EMULSIONS PREPARED WITH THE KNOWN EMULSIFYING AGENTS OF CREAM*

HILDA F. WIESE AND L. S. PALMER

Division of Agricultural Biochemistry, University of Minnesota, St. Paul, Minnesota

The nature of the material adsorbed on the surface of the fat globules in cow's milk has been the subject of a number of investigations. An excellent review of the literature to 1925 is given by Hattori (1). With one exception (2), all the researches have been concerned with the identification of an adsorbed protein. Since the latter compounds are known to be good emulsifiers, it is not surprising that each of the major proteins found in cow's milk should, at one time or another, have been assumed to constitute the active fat stabilizer. Different methods of isolation, however, have yielded products varying in composition and properties. The results published, therefore, have not been uniform. The active fat stabilizer has been reported to be a glycoprotein (3), casein (4), albumin (5), a mixture of a globulin-like protein and phospholipides (2), and a new protein (1) differing in composition from any of the other known milk proteins.

In order to ascertain whether some of these materials might constitute the sole fat stabilizer in cream, artificial emulsions of butterfat stabilized by various milk constituents were prepared. Since this investigation was concerned only with the fat emulsion of cream and the churnability of cream is the most characteristic reaction of the fat component, each of the artificial emulsions was subjected to the churning test.

In preparing the butterfat emulsions, sufficient melted filtered butterfat to give a four per cent milk was added to a one per cent dispersion of the emulsifier at 100° F. This was passed through a Gaulin homogenizer, at such a pressure as to yield a butterfat dispersion resembling microscopically as nearly as possible that of whole milk. The best emulsions were usually obtained at a pressure of 400–500 pounds. The milk was separated in a McCormick-Deering hand separator, operating at normal speed, the cream standardized with its own skimmilk to 30–35 per cent butterfat, cooled over night at 40° F., churned at 55°–57° F. in a glass Dazey churn

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of suitable size, at a crank speed of 90–120 r.p.m. (320–430 r.p.m. of the paddle), and a microscopic examination of the butter made. Each butter was worked slightly with wooden butter paddles before the microscopic examination was made. The type of emulsion present in the churned product was determined by the microscopic method devised by Palmer (6). When the water droplets in a water-in-oil emulsion are in focus and the objective is lowered, the water droplets show bright centers just before they disappear. With oil-in-water emulsions, the oil globules show bright centers when the objective is raised.

The same general procedure was followed in the preparation, churning and examination of all the emulsions.

Fresh buttermilk, calcium caseinate, serum globulin, lactalbumin and a crude egg yolk phospholipide were used as the emulsifying agents. In every case a very good dispersion of butterfat in water was obtained. The macroscopic and microscopic appearance of the milks resembled very closely that of whole cow's milk and each one was stable after separation. However, there was considerable difference in the ease of separation and churnability of the creams.

BUTTERFAT DISPERSED IN BUTTERMILK

According to the foam theory of churning, (7) the foaming compound in milk and the fat stabilizing substance are identical and remain incorporated in butter as a rigid, solid, continuous phase of dehydrated colloid. The fat emulsifying agent therefore would not appear in the buttermilk. On the other hand, if cream is considered an oil-in-water type of emulsion and butter a water-in-oil type, one must assume an inversion of phases taking place at some stage of the butter making process. The fat stabilizing material, then, must be removed from the globules during the churning process to permit coalescence of the fat to form the continuous phase in butter. Although some of the material adsorbed on the surface of the globules might remain incorporated in the water droplets in the butter, at least part of it should appear in the buttermilk. In view of these contradictory theories of churning, it seemed desirable to determine whether buttermilk contained materials which would stabilize a butterfat emulsion comparable in all respects to the butterfat emulsion of normal cow's milk.

For this purpose, melted filtered butterfat and fresh buttermilk at 100° F. were passed through a Gaulin homogenizer, separated, cooled and churned according to the procedure described above. The milk separated and churned very well. Microscopic examination of the cream showed no clustering of the fat globules. A small amount of foam was produced during the churning process. Butter formed and gathered in a normal way in 27 minutes. There was nothing abnormal about the appearance or behavior of the milk. In view of the results obtained with other emulsions, it ap-

pears probable that the buttermilk used as the dispersions medium contained some of the same material which stabilized the fat globules in the original whole milk. However, the material had not been denatured or altered in such a way as to destroy its physical-chemical emulsifying property.

BUTTERFAT DISPERSED IN CALCIUM CASEINATE SOL

The casein used in the preparation of the calcium caseinate milk was grain-curd casein which had been reprecipitated from ammonium hydroxide solution, rinsed, washed with isoelectric distilled water by soaking in new wash water for exactly one week, and then extracted with alcohol and ether. The casein was ground with an excess of calcium hydroxide, the excess alkali removed by precipitation with oxalic acid, and the mixture supercentrifuged. Two per cent phosphoric acid was slowly added to the supercentrifuged material by means of a capillary pipette and mechanical stirrer until pH 6.47 was reached.

Microscopically the butterfat dispersion prepared at a pressure of 300–400 pounds with the calcium caseinate-calcium phosphate stabilizer was very good. However, considerable difficulty was experienced in separating the milk. The emulsion showed no tendency to break but a thick, almost solid, cream collected between the discs of the separator, clogging the openings and preventing any liquid cream from flowing out. By scraping the thick cream from the discs and bowl of the separator and mixing it in a mortar with its own skimmilk, a stable uniform cream was again readily prepared.

During the churning process a coarse foam which completely filled the churn formed at once. The foam became finer as the crank speed of the churn was increased from 90 to 120 r.p.m. Butter granules formed but did not gather even after several hours of churning. Microscopic examination of the foam showed partially churned material, that is, material in which very large fat globules and irregular masses of fat containing minute water droplets, and a regular dispersion of fat-in-water were visible.

Various amounts of lactic acid were then added to fresh portions of the cream and the time and ease of churning noted.

The addition of lactic acid reduced the time of churning and aided in the gathering of the butter granules. The fact that most of the fat globules in the second cream were clustered did not reduce the churning time below that of the fourth cream in which there were no clusters present. Although the butter granules gathered in the case of all the acid creams, they did so very slowly and a great deal of fat was lost in the buttermilk, *i.e.*, 6–7 per cent. Increasing the acidity to pH 4.6 theoretically should decrease the stability of the butterfat emulsion, for the surface activity of the adsorbed casein would be at a minimum at the isoelectric point. In general, this was

found to be true. The time of churning, taken as a measure of the stability of the emulsion, decreased with an increase of acidity.

The stability of the emulsion, although lessened, was not decreased to the breaking point during the increase in acidity which passed through the isoelectric point. There was, no doubt, a reversal of the charge on the surface of the globules between pH 5.2 and 2.2. The cream at pH 2.2 foamed only slightly. The production of an excessive amount of foam which decreased markedly before butter granules formed was characteristic of all the other calcium caseinate creams. This appears to be in contradiction to Rahn's theory that foaming is essential for churning. The cream yielding the smallest amount of foam churned the quickest. None of the churnings, however, resembled those of normal cream. The results of the churning experiments are given in table 1.

TABLE 1

Churnability of calcium caseinate cream

FAT CONTENT OF CREAM	CHURNING TEMPERATURE	CHURNING TIME	pH OF BUTTERMILK	FAT IN BUTTERMILK
Per cent	°F.			*Per cent*
22.5	52.8	2 hrs. 30 min.*	.....	.....
22.7	57.2	2 hrs. 10 min.	6.0	6.0
32.0	57.2	1 hr. 20 min.	5.2	.....
27.0	56.3	35 min.	2.2	7.0

* Butter granules did not gather.

BUTTERFAT DISPERSED IN LACTALBUMIN SOL

The lactalbumin solution was prepared from a sample of soluble lactalbumin powder[1] obtained from the Research Laboratories, Bureau of Dairying, Washington, D. C. The milk prepared by dispersing butterfat in the lacalbumin solution separated much more easily than the calcium caseinate dispersion, but not as well as the fresh buttermilk dispersion. After standardization, no clusters were visible microscopically. Churnings were also made of the cream to which varying amounts of lactic acid were added. During churning a large volume of coarse foam appeared at first. This gradually became finer, decreasing in volume and becoming a uniform whip before butter granules formed. The churned products showed typical butter structures, although there was considerable difference in the degree of dispersion of the water droplets. All the products were very light in color, the most acid cream yielding a perfectly colorless product. The latter contained extremely small water droplets. This observation for an artificial cream is in accord with the well known fact that the color of butter is, in

[1] The lactalbumin powder contained 35.37% protein (N × 6.38), 0.195% ash and 64.44% lactose (by difference). We wish to acknowledge the kindness of Dr. L. A. Rogers, who furnished us with this product.

part, dependent on the distribution of the water in the butter. The churning results are given in table 2.

TABLE 2

Churnability of lactalbumin cream

FAT CONTENT OF CREAM	CHURNING TEMPERATURE	CHURNING TIME	pH OF BUTTERMILK	FAT CONTENT OF BUTTERMILK
Per cent	°F.		*Per cent*	*Per cent*
36.5	57.2	32 min.	6.66	4.3
36.5	57.2	33 min.	4.85	3.4
27.5	57.2	35 min.	3.24	2.5

BUTTERFAT DISPERSED IN GLOBULIN SOL

Since lactoglobulin and serum globulin have been shown to be identical (8) (9), a globulin dispersion was prepared by dispersing commercial boval[2] in water containing a small amount of ammonium sulphate. After supercentrifuging, the globulin was precipitated with half saturated $(NH_4)_2SO_4$, again supercentrifuged, redispersed in water and reprecipitated. The globulin dispersion was then dialyzed in cellophane bags against running tap water until almost free from sulphates.

The globulin milk separated better than the calcium caseinate milk but not well enough to be considered normal. It yielded a heavy cream, some of which collected between the discs of the separator. It remained stable, however, and mixed readily with the thinner portion and was then standardized to 35 per cent butterfat. During the churning process, a coarse foam formed at the beginning quite similar to that produced by the casein creams. One cream, which required an hour and 43 minutes to churn, behaved more like a casein cream than any of the others. Butter granules formed on the sides of the churn long before churning was complete. The butter structure was poor and the loss of fat in the buttermilk was about 15 per cent. All the other churned products had a typical butter structure but were extremely pale in color due to the presence of very fine water droplets. The difference in the behavior of the cream at pH 5.27 cannot be explained. Acidification had little effect on the churning time of the other creams. The churning experiments gave the results shown in table 3.

BUTTERFAT DISPERSED IN PHOSPHOLIPIDE SOL

The phospholipide content of cow's milk has been investigated by a number of workers, the amount reported varying from .040 to .1173 per cent. According to Glikin (10) the major part of it passes into the cream but when the cream is churned, only a trace is retained in the butter (11) (12). Most of it appears in the buttermilk. Palmer and Samuel-

[2] Dried blood serum.

TABLE 3
Churnability of globulin cream

CHURNING TEMPERATURE	CHURNING TIME	pH OF BUTTERMILK	FAT CONTENT OF BUTTERMILK
°F.			*Per cent*
56	27 min.	6.84	5.0
57	1 hr. 43 min.	5.27	15.0
57	30 min.	4.19	4.4
57	30 min.	3.40	5.0

son (2) also found phospholipide-like substances in the material isolated by them from the fat globules in washed cream. It appears likely, therefore, that a part of the phospholipide in milk is closely associated with the fat globules. With this in mind, a butterfat dispersion stabilized by phospholipide was prepared.

Since the amount of phospholipide in cow's milk is so small isolation from this source was impractical. A more feasible procedure was to secure the phospholipide from a product rich in lecithin. While egg yolk lecithin might differ from the mixed milk phospholipides in chemical composition insofar as the fatty acids are concerned, it still possesses the property of an excellent emulsifying agent. Crude phospholipide was prepared from fresh egg yolks. The water, fat, sterols and other acetone soluble substances were removed from the yolks by repeated extraction with acetone until the extract was practically colorless. The moisture-free, fat-free, sterol-free residue was extracted with hot 95 per cent ethyl alcohol and the solvent distilled off under reduced pressure. The crude phospholipide was recovered from the residue. A one per cent aqueous dispersion served as the emulsifying agent

The phospholipide milk separated more nearly like natural cow's milk than any of the other butterfat dispersions prepared. The cream, however, did not give normal churnings. Butter granules formed but did not gather well, about ten minutes being required for the latter process. The butter did not "break." Microscopically, two of the products showed peculiar structures with very small water droplets dispersed in fat together with many fat globules still intact, closely packed and massed together. The best product microscopically was obtained at pH 4.75.

It was apparent in two of the products that the inversion of the oil-in-water type of emulsion was not complete. The phospolipide appeared very effective as an oil-in-water stabilizer. According to Feinschmidt (13), Fugii (14), Price and Lewis (15) and Remesow (16), the isoelectric range for lecithin lies between pH 2.2–6.3. Although the churnings were made in this range where lecithin was exhibiting its minimum surface activity, the

emulsions were not completely inverted. The results of the churning experiments are given in table 4.

TABLE 4

*Churnability of phospholipide cream**

CHURNING TEMPERATURE	CHURNING TIME	pH OF BUTTERMILK	FAT CONTENT OF BUTTERMILK
°F.			*Per cent.*
54	30 min.	6.69	2.9
56	25 min.	4.75	1.9
57	60 min.	3.10	About 12.0

* Fat content of cream was 31 per cent.

BUTTERFAT DISPERSED IN GLOBULIN AND PHOSPHOLIPIDE SOL

In the preceding experiments, only single substances, calcium caseinate, lactalbumin, serum globulin, or egg yolk phospholipide were used as the fat stabilizer. It seemed possible that combinations of these would yield emulsions which behave more like normal cow's milk both on separation of the cream and churnability of the cream than those stabilized by one constituent. One combination only was tried. Palmer and Samuelsson (2) concluded that protein and phospholipide existed in a ratio of approximately 1:6 in the "membrane" material isolated by them. The skimmilks from the globulin and phospolipide creams were therefore mixed in the ratio of 1:6 and melted filtered butterfat dispersed in the mixture.

The milk separated well but again the acid cream failed to yield typical churned products. The microscopic structure of the products was slightly better than those of the phospholipide cream. The results of the churning experiments are given in table 5.

"WASHED" CREAM

Storch (3) was the first to observe that cream would churn normally when obtained by diluting milk with water and passing it through a centrifugal cream separator. He observed that the stability of the cream was not lessened by repeating the washing 4 times using 4 volumes of water to 1 of cream at each washing. Palmer and Samuelsson (2) verified these observations, starting with fresh cream instead of whole milk.

We have again confirmed Storch's findings, using even more drastic manipulation. We have repeatedly prepared a stable cream which has been freed from milk plasma by dilution and reseparation. Four volumes of distilled water, temperature about 100°–110° F.,[3] to one volume of cream were employed at each dilution and the number of such dilutions repeated as many as 8 times without destroying the cream emulsion. Sepa-

[3] A wash-water temperature of about 105°F. seemed to give the best results.

TABLE 5.

*Churnability of globulin-phospholipide creams**

CHURNING TEMPERATURE	CHURNING TIME	PH OF BUTTER MILK	FAT CONTENT OF BUTTERMILK	GENERAL AND MICROSCOPIC APPEARANCE OF PRODUCT
°F.			*Per cent*	
57	5 min.	6.6	3.6	Yellow in color. Fat continuous phase. Wide distribution in size of water droplets.
57	17 min.	4.2	3.7	Light yellow in color. Fair distribution of water-in-fat. All water not in droplet form.
56	20 min.	3.9	5.6	Light yellow in color. Wide distribution of water droplets. All water not in droplet form.
57	47 min.	3.4	8.0	Light yellow in color. Fair distribution of water droplets. All water not in droplet form.

* Fat content of cream was 35 per cent.

ration of the cream was effected in each instance in the present studies by means of a Sharples supercentrifuge geared down to operate at a speed of about 10,000 r.p.m. A volume of wash water from the 4th to 8th washings equal to one and one-half times the volume of original cream, gave either a negative or faint biuret test on evaporation to dryness.

The churnability of the creams prepared in this manner was tested in each case in the following manner. The washed cream was standardized to a fat content of 35 per cent using distilled water, the cream from the final washing usually exceeding this fat percentage. The standardized cream was tempered over night at a temperature of 38°–40° F. and churned the following morning at temperatures ranging from 44°–55° F., depending on the season. The churn employed was either a 1 or 2 quart glass Dazey churn or a small metal barrel churn when larger volumes of washed cream were obtained.

Inasmuch as these churnings were carried out for another purpose, the results of which will be reported in a later paper, the data on these churnings are not as complete as might be desired. The available data are summarized in table 6. In addition certain interesting observations may be mentioned.

Although the cream prepared by washing is a stable emulsion having both the macroscopic and microscopic appearance of normal cream, its stability is somewhat less than normal unwashed cream in that a small amount of churning takes place in the passage of the diluted cream through the centrifuge, particularly after the 4th washing. This stability is further decreased by increasing the acidity, as seen in experiment 2, table 6. This cream showed a much greater tendency to churn during its washing and butter began to form at once in the churn.

TABLE 6

Churning experiments with washed cream

EXP. NO.	VOLUME ORIGINAL CREAM	CONDITION OF ORIGINAL CREAM	NUMBER OF WASHINGS	FAT CONTENT WASHED CREAM	FAT CONTENT CHURNED CREAM	TIME REQUIRED FOR CHURNING	COMPOSITION BUTTER MILK
	liters			*per cent*	*per cent*	*minutes*	
1	1.0	fresh-sweet	8	49.0	35	20	0.36% T.S.
2	1.0	0.45% acidity	6	.....	35	at once	1.77% T.S.
3	3.8	fresh-sweet	6	.....	35	30	.....
4	8.0	fresh-sweet	6	.....	35	slowly	.....
5	5.7	fresh-sweet	6	31.0	31	readily	.....
6	15.4	fresh-sweet	6	56.0	35	readily	.....
7	15.4	fresh-sweet	6	49.5	35	readily	.....

Special attention was given to the microscopic structure of the butter obtained in experiment 4, in which the butter formation was not as rapid as in the previous experiments. The structure was, however, entirely normal, showing the microscopic water drops well dispersed in the continuous fat phase.

The buttermilk from the churning in experiment 6 was studied microscopically both before and after passing it through the Sharples supercentrifuge at high speed (30,000 r.p.m.). Before centrifuging considerable fat still in globular form was observed, as well as some butter particles, showing that the churning had not been very exhaustive. This was true for most of these experiments but cannot be employed as criticism because they were not planned from this point of view. After supercentrifuging the buttermilk, practically no fat could be observed microscopically. In experiment 7, however, the churning must have been quite exhaustive because practically no fat was thrown out in the bowl when the buttermilk was supercentrifuged at high speed.

SUMMARY

Fresh buttermilk contains a substance or substances having the ability to stabilize oil-in-water emulsions. It is probable that a portion of this material comes from the surface of the fat globules in cream during the

churning process. The butterfat-in-buttermilk dispersion resembles whole milk in every respect, in general appearance, microscopic structure, cream separation and churnability of the cream.

Stable emulsions of butterfat can be prepared in dispersions of calcium caseinate, lactalbumin, globulin and phospholipide. These resemble whole cow's milk in general appearance, formation of a cream layer and microscopic structure. Each shows an abnormal behavior either on separation of the cream or in the churability of the cream. The best separation is obtained with a phospholipide stabilized emulsion and the best churning with a lactalbumin stabilized emulsion.

Since cream freed from milk plasma churns normally, it seems justifiable to draw conclusions from the emulsions which likewise lacked most of the constituents of normal milk plasma. It is apparent from the preceding churning experiments that no one of the milk constituents used produces emulsions which behave entirely like normal cow's milk. It, therefore, does not appear probable that any one of the substances tested constitutes the sole material adsorbed on the surface of the fat globules in normal cow's milk.

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METHODS FOR USE IN THE BACTERIOLOGICAL EXAMINATION OF DRY MILK AND RELATED POWDERS

FOREWORD

The American Dairy Science Association, through its committee on bacteriological methods, is formulating bacteriological procedures useful in controlling the quality of dairy products. This committee is acting through sub-committees appointed from men in the organization who have had experience with the bacteriological analysis of various dairy products. Obviously, the formulation of such methods should not be left to the arbitrary decision of a committee, but should be the result of suggestions and criticisms coming from all interested parties, whether or not they are members of the American Dairy Science Association. The committee, therefore, wishes to serve as a center about which methods satisfactory to the largest number may be evolved.

The purpose of this preliminary report is to submit an outline of methods for making bacteriological examinations of dry milk and certain related powdered baby foods. These methods will be revised later in accordance with the suggestions received before they are finally adopted by the American Dairy Science Association and included in a general report on bacteriological methods of analyzing dairy products.

METHOD OF SAMPLING

It is very important that all utensils and containers used for sampling milk powder be clean, dry and sterile, and that the work be done as speedily as possible. Milk powder absorbs moisture from the atmosphere very rapidly. Samples should always be taken at points beneath the exposed surface.

If samples are to be taken from a bulk or mass of powder not contained in standard packages it is convenient, with the aid of a sterile tablespoon or wide spatula, to obtain samples of substantially the same size from various points throughout the powder mass. Such samples are to be combined in a suitable sterile container from which a properly mixed composite sample is obtained later.

Sampling of milk powder from standard small package units may be obtained simply by withdrawing the desired amount from the powder avoiding that immediately adjacent to the exterior surfaces. A sterile tablespoon or sterile spatula is very convenient for this purpose.

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If samples are to be obtained from the standard bulk packages such as barrels, drums or boxes containing 25 pounds or more it is desirable to remove the layer of powder adjacent to the barrel head or opening to the depth of about three inches and insert in such a cleared area a sampling tube or device similar to an elongated butter or cheese trier. Such a device may not remove an unbroken column of milk powder but in general a representative sample obtained through the entire depth of the container can be secured by such a device, the efficiency of which is determined largely by its design and the manipulations involved in its use. At least three such "plugs" or columns of powder should be withdrawn from a single container. The number of samples thus obtained from a single lot or consignment will obviously be determined by the requirements to be met.

When it is necessary to prepare a composite sample from the combined portions obtained as above in order that the final sample may be representative of a given quantity of powder, several good sized samples obtained from the various batches should be collected in a container of suitable size in order to permit thorough mixing of the desiccated product in order that the sample to be withdrawn for analysis may be representative of the combined portions. Single or double friction top cans, previously cleaned, and sterilized are recommended for collecting, holding or shipping samples. Variations which may occur in a given quantity of powder can be more readily detected by examining separate samples rather than a composite sample, and, in some cases, this practice is preferable.

PREPARING THE SAMPLE FOR ANALYSIS

If the sample of powder should completely fill the container in which it is collected or received, the entire contents should be transferred to another clean, dry, sterile container of ample size to permit proper mixing. The detailed manipulations for mixing the sample may be left to the discretion of the individual, it being understood that the necessity of uniform mixture is as desirable in this case as in any other wherein a representative sample is desired. It is important during the mixing and handling of the product to prevent extraneous contamination as from a dusty atmosphere, improperly cleaned or unsterilized utensils. The same general precautions as are applied to the preparation of any sample for bacteriological analysis apply also to dry milk.

SUGGESTED METHODS OF ANALYSIS

(*a*) Standard Agar Plate Method.

The standard agar plate method of the American Public Health Association as used for fluid milk may be used for determining the number of organisms in dry milk.

Preparing Dilutions:

It is believed desirable to follow as closely as practical, methods already recognized as standard procedures. For this reason the method of bringing the volume or weight up to 100, as used in the Standard Methods of Milk Analysis of the American Public Health Association, is preferred to the method of adding the material to be tested to 100 cc. or grams as the case may be.

For the one to ten dilution completely dissolve or mix ten grams of the prepared sample in ninety grams of distilled water. In certain instances it may be more convenient to prepare a one to ten dilution by using forty-five grams of water and five grams of powder. If a one to hundred dilution is desired, this may be prepared from the one to ten dilution or one gram may be used with ninety-nine grams of distilled water. The dilutions may be conveniently prepared by weighing the dry sample directly into a bottle containing the dilution water; or into the empty sterile dilution bottle, the required amount of sterile water being added just prior to dissolving the powder for plating. If the latter procedure is followed glass stoppered bottles will be necessary in order to sterilize the bottles by hot air. In the examination of milk powders or compounded infant foods which do not readily form a complete solution with water, it is desirable to facilitate such solution by bringing the temperature of the dilution water to 110–120° F. before mixing with the sample. The plating should be completed within 15 minutes after addition of the water in order to prevent growth in the dilution bottle which would affect the final results.

If lactic acid milk powder preparations are being examined a dilution may be made by using N/10 LiOH as the diluent in order to aid in dissolving the powder. This same type of diluent may be also used for dilutions for the bacteriological examination of powders containing calcium caseinate, although in this case a one to hundred dilution in distilled water is preferable in order to prevent excessive foaming.

In the case of incomplete solution of the milk powders or certain constituents of compounded preparations, the diluted product should be kept thoroughly agitated during withdrawal of the sample with the pipette in order that a uniform sample of the solution and suspended material may be transferred to the petri plate.

Preparing Plates:

Dilutions should be made which will give not more than 300 nor not less than 30 colonies on the plate. The preparation of the media and pouring of the plates should follow the suggestions outlined in the latest edition of the Standard Methods of Milk Analysis of the American Public Health Association (Fifth Edition, 1928).

Incubation Temperature:

In addition to the incubation temperature of 2 days at 37° C. as recommended in the standard procedure, other incubation periods and temperatures may be used for special purposes, such as 5 days at 25° C., 3 days at 30° C., 2 days at 35° C. or 2 days at 55° C. The later temperature is especially useful for estimating the number of thermophilic bacteria which are often present in large numbers due to certain steps in the manufacturing process.

Counting of Plates—Precautions:

Extreme precaution should be exercised in counting the incubated plates in order to differentiate between the bacterial colonies and suspended constituents of the sample transferred to the plates from the initial dilutions. Insoluble milk powders or compounded products containing insoluble material frequently cause residues in the plates which are readily confused with bacterial colonies. Devices for counting the plates with the aid of artificial illumination and low magnification are strongly recommended. Doubtful objects should be examined with higher magnification to determine whether they are colonies.

(*b*) Microscopic Colony Count (Frost Method).

In laboratories already equipped and experienced in counting bacteria in milk by this method, it would seem that this method could be readily used in the routine control of dried milk. Directions as outlined in the Standard Methods of the American Public Health Association should be followed.

The original sample should be diluted as suggested in the methods for the agar plate count. Incubate for 12 to 16 hours at 37° C.

(*c*) Direct Microscopic Count (Breed Method).

The direct microscopic count applied to milk powders has a certain value for indicating the bacterial character of the fluid milk from which the product was made. Where this method is to be used for purposes of information of this kind it should be carried out according to the directions given in the Standard Methods of Milk Analysis of the American Public Health Association.

The direct microscopic count cannot be relied upon as an adequate procedure for determining the bacterial count of milk powders. It may be of supplementary value in conjunction with certain plating procedures listed herein.

(*d*) Examination for Hemolytic Streptococci.

Standard nutrient agar plates to which has been added 0.5 per cent sodium chloride and 2 to 5 per cent defibrinated blood is suggested for use

in determining the presence of hemolytic streptococci. The blood should be added aseptically after the agar is melted and cooled prior to pouring the plates. The plates should be incubated for 2 days at 37° C. Examination should be made at the end of 24 to 48 hours.

Suspicious hemolytic colonies should be examined by Gram staining to determine the presence of streptococci. Before definite conclusions can be drawn, reference should be made to standard text books on bacteriology dealing with the differentiation and recognition of hemolytic streptococci.

If conditions permit its use, veal infusion medium will give somewhat better results than the standard nutrient agar for the growth of the hemolytic streptococci. This medium is prepared as follows:

Ground lean veal	500 grams
Distilled water	1,000 cc.

Infuse over night in a cold room. Strain through cheese cloth by pressing. Make up to the original volume. Bring to boiling with frequent stirring and boil until the infusion is clear and the coagulum brown. Strain through cheese cloth and filter through paper. Adjust reaction to pH 6.8 to 7.0. Add 0.5 per cent Difco peptone and 0.5 per cent sodium chloride. The broth should then be autoclaved for 15 minutes at 20 pounds' pressure. This is a higher pressure than that used for the final sterilization and is used in order to avoid precipitates in the finished product. Egg may be added previous to autoclaving if a glass clear medium is desired. Filter and sterilize at 15 pounds' pressure for 15 minutes. Before the final sterilization, 1.5 per cent agar is added and the medium distributed in flasks in readiness for plating. Blood (rabbit, horse, sheep, etc.) may be added aseptically just prior to plating and after the agar has been melted and cooled to 50 degrees C.

(*e*) Examination for Thermophilic Bacteria.

For the routine determination of the presence of thermophilic organisms plates may be prepared from standard nutrient agar. For investigation or research purposes it is recommended that the following materials be added to each 1,000 cc. of standard agar.

Bacto Trytophane Broth	2.5 grams
Bacto Yeast Extract	1.0 gram
Dextrose	1.0 gram
pH	7.0 ± 0.2

The plates should be incubated for 48 hours at 55° C. At least 15 cc. of medium should be used in each plate to prevent excessive drying during the incubation. Water should be placed in the incubator in order to reduce the drying of the plates to a minimum.

MEDIA FOR SPECIAL PURPOSES OTHER THAN THOSE LISTED ABOVE

(*a*) Inasmuch as the standard procedure of the American Public Health Association as outlined above (*a*) may not be conducive to the growth of all organisms in milk powder any more than it is to the growth of all organisms in natural fluid milk, the following medium is suggested for special purposes.

Bacto Trytophane Broth	2.5 grams
Bacto Peptone	2.0 grams
Bacto Yeast Extract	1.0 gram
Dextrose	5.0 grams
Agar	15.0 grams
Water	1,000 cc.

Incubation temperature in this case should be for 3 days at 30° C. or at other times and temperatures in accordance with the results desired.

(*b*) Whey agar may also prove satisfactory for obtaining counts required for investigational or interpretative purposes and may be prepared according to methods outlined in Suggested Methods for the Microbiological Analysis of Butter, JOUR. OF DAIRY SCI., Vol. 13, 396–397, 1930.

EXAMINATION FOR YEASTS AND MOLDS

The determination of yeast and mold counts in dried milk, while not a common practice, is used quite extensively in the butter industry. No doubt a more complete study of dried milk from this view-point might give us some valuable information regarding sanitary conditions. (See Suggested Methods for the Microbiological Analysis of Butter, JOUR. OF DAIRY SCI., Vol. 13, 380–405, 1930.)

METHOD OF REPORTING COUNTS

Results should normally be reported per gram of dry powder. If desired they may be transformed to the fluid basis by dividing by the factor used in reconstitution. This is usually 8 parts of water to 1 gram of powder.

In the interest of uniformity of practice and interpretation of data from various sources, the medium used and the time and temperature of incubation should always be reported in direct conjunction with the numerical results obtained on the one gram basis. It is desirable that this procedure be followed when the standard medium and incubation are used as well as in those cases wherein special media and variable incubation periods and temperatures are used.

CONCLUSION

The views expressed in this report are those of a committee appointed by the American Dairy Science Association. As such they are printed for

the general criticism of other members of the American Dairy Science Association and other interested parties.

Separate copies of this report may be secured at cost from the chairman of the Committee on Bacteriological Methods.

Sub-Committee on Bacteriological Methods
of Examining Condensed, Evaporated, and
Dry Milk:

P. A. DOWN, *Chairman*
G. C. SUPPLEE
P. S. PRICKETT
G. J. HUCKER

Committee on Bacteriological Methods:

R. S. BREED, *Chairman*

NOTE ON USE OF ALKALINE WATER BLANKS FOR PLATING DRIED MILK PRODUCTS

PAUL S. PRICKETT AND NORMAN J. MILLER

Bacteriological Laboratory, Mead Johnson & Co., Evansville, Ind.

In the counting of bacterial colonies in plates prepared from dried milk products considerable difficulty is encountered in distinguishing tiny particles of undissolved protein suspended in the agar from small colonies of bacteria, especially in the powdered acid milk products. Due to this difficulty there are times when erroneous results are reported.

Because of this difficulty, a study was made of methods to eliminate the protein particles without materially affecting the results of the plate count of the product. Since milk proteins are dissolved by alkaline solutions, the effects of different, individual alkalies and various combinations of them were tested for their ability to dissolve the protein particles insoluble in distilled water. More than twenty different alkaline solutions, including the sodium phosphates, were tested upon various kinds of dried milk products. The best results were obtained with lithium hydroxide solutions. Further work showed that M/5 and M/10 LiOH solutions were satisfactory for this purpose. A lack of germicidal action of these LiOH solutions was shown by a comparison of bacterial counts of various powdered milk products obtained by using distilled water dilution blanks and M/5 and M/10 LiOH dilution blanks.

Gray (1), in his study of the action of various inorganic salts on bacteria, showed that apparently some organisms retain their viability unimpaired after incubating 48 hours in media containing as much as 3.3 per cent lithium salts. He also found that all of the organisms tested, with the possible exception of the cholera vibrio, were unaffected in viability by 48 hours of incubation in media containing 0.8 per cent lithium salts.

In the present study a 0.48 per cent or M/5 LiOH solution was the strongest concentration used for dilution blanks. Furthermore, the organisms are in contact with a solution of this strength for only a short interval,[1] before the concentration of lithium salts is greatly decreased through the addition of agar in the pouring of the plate.

Although at this time detailed work with these alkaline blanks has not been entirely completed, sufficient data have been accumulated to show that

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[1] These comparative counts were obtained by following Standard Methods of Milk Analysis (2) which recommend a maximum interval of 15 minutes between preparing the dilution and pouring the agar.

counts obtained using M/5 and M/10 LiOH dilution blanks compare very favorably with parallel counts obtained using distilled water dilution blanks. When a discrepancy occurs it is usually with a powdered acid milk product, the higher counts being obtained when the alkaline blanks are used. This is probably due to the inhibitory effect of a lowered pH in the plates seeded with inocula prepared in distilled water dilution blanks. In some cases this pH is found to be as low as 4.5, which no doubt inhibits colonial development of some of the bacteria present. On the other hand, the pH of the inoculum-medium mixture in the plate is raised to approximately 7.0 when dilutions of the same acid products are prepared with M/5 LiOH dilution blanks.

With a powdered acid milk product whose pH is low, M/5 LiOH dilution blanks seem to give the best results (probably because the acid must first be neutralized before the water-insoluble protein particles can be dissolved), while for a powdered milk product with a higher pH the M/10 LiOH dilution blanks are satisfactory. Excessive shaking of powdered milk dilutions prepared with LiOH solutions should be avoided as the fat has a greater tendency to be churned out in these dilution blanks than with the distilled water dilution blanks.

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STUDIES ON LACTOBACILLUS THERMOPHILUS

DAVID B. CHARLTON

Department of Bacteriology, Oregon State College
Corvallis, Oregon

In a review of the literature on the subject of thermophilic and thermoduric bacteria, Robertson (4) stated, "Only one non-spore-forming thermophile, *Lactobacillus thermophilus* Ayres and Johnson, has been isolated." Although thermophilic bacteria have been studied extensively in recent years, it appears that *Lactobacillus thermophilus* has not been encountered since the original isolation. It is therefore pertinent that an additional isolation of the organism be reported and a more complete description presented. As in the original isolation the organism was secured during an investigation of high plate counts with "pin point" colonies from pasteurized milk.

PRELIMINARY OBSERVATIONS

In the routine bacteriological analyses of the Corvallis milk supply, there was noticed a higher bacterial count in the pasteurized milk from one of the local plants, than in the raw milk delivered to the plant. The 1:100 dilution plates on the pasteurized milk were crowded with minute colonies. Daily platings were then made and the plates were incubated at 37° C., with a duplicate set at 50° C. Every sample of the pasteurized milk tested showed thermophilic bacteria which were present in widely varying numbers. Growth at 37° C. was poor in that the colonies were small and sometimes did not appear. At 50° C. the colonies were larger, and by the use of milk powder agar, distinct colonies averaging 0.8 mm. in diameter were obtained. The milk revealed rod forms on direct examination. At the time the preliminary work was being carried on, it was reported from the milk plant that the milk after pasteurization had an acidity of 0.25 per cent calculated as lactic acid, that patrons had complained of a peculiar flavor in the milk, and that a slight odor could be detected in the pasteurizing vat.

DESCRIPTION OF LACTOBACILLUS THERMOPHILUS

A number of cultures of the organism were isolated from colonies on the milk powder agar plates incubated at 50° C. Stock cultures were maintained by making daily transfers in litmus milk, incubating at 50° C. for approximately twenty hours. The same time and temperature were used throughout this work unless otherwise stated.

Morphology

Form and size. Non-spore-forming rods, averaging 1.0 by 3.5 microns in milk culture. Variations from 2.5 to 6 microns in length are observed. In broth, the cells are somewhat longer. The rods are straight or slightly curved.

Arrangement. Single cells and short chains predominate in milk cultures. In broth, chains occur most frequently, some being very long.

Motility. Non-motile.

Staining reactions. Gram positive, but commonly gram negative in milk culture. The cells are not stained uniformly with methylene blue.

Cultural Characteristics

Agar colonies. On a satisfactory medium (such as milk powder agar), surface colonies are slightly raised, glistening, translucent, circular with a curled edge and vary from 1 to 2 mm. in diameter. Sub-surface colonies are filamentous. Their diameter and density of structure depends upon the medium.

Agar slant. Filiform, glistening, translucent, flat or slightly raised growth on milk powder agar.

Broth. Slight growth in plain broth. In broth rich in peptone, good growth takes place. No surface growth, no sediment, moderate clouding.

Litmus milk. Acid with partial reduction of the indicator.

Biochemical Features

Indol—not produced.

Nitrates—not reduced.

Action on carbohydrates and alcohols. Glucose, galactose, levulose, mannose, lactose, maltose, sucrose, raffinose, soluble starch and dextrin fermented with the production of acid, but no gas. Arabinose, xylose, glycerol, rhamnose, salicin, insulin, and mannitol not fermented.

Oxygen relationships. Organism facultative; in agar-shake cultures the best development of colonies takes place near the surface of the medium.

Temperature relationships. Slight or no growth at 30° C. Weak growth at 35° C. (On plates at this temperature only a small part of the total inoculated will grow compared with duplicates at 50° C.) Optimum 50° to 60° C. Maximum at approximately 62° C. A milk culture is rendered sterile on heating at 70° C. for thirty minutes.

Total and Volatile Acid

The total and volatile acids were determined in milk cultures of the organism after incubation for 48 hours at 55° C. The total acid was determined by titrating 10-gram samples with 0.1 N. NaOH using phenolphthalein as an indicator, and calculating the acidity as lactic acid. A large number of determinations have all shown from 0.35 to 0.42 per cent. The volatile acid was determined by steam distillation of a 250-gram sample of the milk, after acidifying with 15 ml. of N. H_2SO_4. One liter of distillate was collected and titrated with 0.1 N. NaOH using phenolphthalein as an indicator. The following milliliters of 0.1 N. NaOH, 14.5, 15.6, 10.9 and 13.0 indicate that comparatively little volatile acid was produced, as the figures for sterile milk treated in the same manner are from 3 to 6.

Determination of the Non-Volatile Acid

It is apparent that the non-volatile acid makes up most of the total acid. Zinc salts were prepared from the residues remaining after the steam distillations, using the method by Hammer (3). The results obtained in the study of these salts show that the non-volatile acid formed is largely or entirely *d* lactic acid.

Comment upon the Description

The organism described is practically identical to *Lactobacillus thermophilus* Ayres and Johnson, and may be considered as the same species. From the additional characteristics recorded in the description, it would seem that the possible similarity between *Microbacterium lacticum* Orla-Jensen and *Lactobacillus thermophilus* Ayres and Johnson, as was suggested by Robertson (5), does not hold true.

FACTORS AFFECTING THE GROWTH OF LACTOBACILLUS THERMOPHILUS

The conditions of the Standard Plate Count are not particularly favorable for the growth of *Lactobacillus thermophilus*. The temperature of 37° C. is near the minimum for the organism and at two or three degrees lower (temperatures which exist in many routine incubators) normal development does not take place. Secondly, the organism does not grow in slightly acid media. With other conditions favorable, a pH of 6.0 in agar media, determined colorimetrically, prohibits growth. Many batches of agar used in routine work, but not carefully adjusted to a pH of 6.2 or above, have failed to grow the organism. Thirdly, standard nutrient agar is not a very satisfactory medium for the growth of *Lactobacillus thermophilus*. Surface colonies do not appear, nor can appreciable surface growth

be induced by streaking (this is characteristic of many lactobacilli), although on a more nutritive medium surface colonies do appear. The sub-surface colonies on standard nutrient agar are filamentous, and of very thin veil-like structure averaging 0.4 mm. in diameter, and though not "pin-point" in size, are not readily seen in some cases.

A preliminary study was made of the nutritive requirements of *Lactobacillus thermophilus* in regard to the type and amount of peptone. By substituting Bacto-Proteose peptone in standard nutrient agar, in place of Bacto-Peptone, in the usual 0.5 per cent concentration, luxuriant growth is obtained. Surface and sub-surface colonies are from one to two mm. in diameter. Almost as good growth can be obtained by increasing the concentration of Bacto-Peptone to two or three per cent. In regard to the minimal peptone concentrations, 0.1 per cent Bacto-Proteose peptone in washed agar without beef-extract supports the growth of distinct colonies, whereas 0.3 per cent Bacto-Peptone is required to grow visible colonies. Here again, any considerable variation from the standard methods formula might not allow growth of *Lactobacillus thermophilus*. The required nutritive substance is present in peptone, particularly proteose-peptone. The organism is not saccharophilic as are the thermo-tolerant "pin-point" streptococci. The addition of diluted sterile milk to an agar plate culture increases the colony size of *Lactobacillus thermophilus*, but sterile lactose has little or no effect.

VIABILITY OF LACTOBACILLUS THERMOPHILUS

In maintaining stock cultures, it was found that cultures of the organism rapidly became sterile. Milk cultures which had been incubated at 50° C. for 12 to 24 hours and then held at room temperature were sterile in from four to ten days. Cultures containing calcium carbonate remain viable only somewhere longer. Broth cultures and colonies are usually sterile in a week or less. Storing cultures at ice-box temperatures lengthens the period of viability.

THE DEVELOPMENT OF LACTOBACILLUS THERMOPHILUS IN THE PASTEURIZING PLANT

The organism was regularly present in the pasteurized milk from one plant during a period of several weeks. The raw milk received at the plant did not reveal the organism either by plating or direct microscopical examinations. As the plant equipment has often been reported as a source of thermophiles, this possibility was investigated. The pasteurizing vats of the Burrell Spray type were in good condition and attempts to find the organism in the cleaned vats just before use were unsuccessful. Swabbings from the sides and corners of the vat as well as drip water in the bottom did not contain thermophilic bacteria. The failure to isolate the organism

in the raw milk and in the equipment seemed inconsistent with the daily appearance of large numbers of the organism in the pasteurized milk. However, a check-up on the plant operations did explain the problem. The milk was being standardized before pasteurization to the desired butterfat content with the pasteurized cream of the previous day. It was a case of daily inoculation as the data in the table show.

Thermophilic bacteria in milk shown by platings at 50° C.

Mixed raw milk in vat before heating	0 in 0.01 cc.
Raw milk after addition of cream	40,000 per cc.
The same, after heating to 143° F.	60,000 per cc.
" , after holding 45 minutes	100,000* per cc.
The cream used to standardize	1,000,000* per cc.

* Approximate numbers due to low dilutions used.

Since discontinuing the addition of pasteurized cream to standardize the milk, weekly platings of the pasteurized milk have been continuously free from *Lactobacillus thermophilus*. The trouble was therefore due to re-pasteurization, often recognized as a source of high counts of thermophilic bacteria. The unusually large numbers in the cream, which gave a sizable inoculation to each vat of milk in the standardizing process, can be explained. The cream was obtained by separating the pasteurized milk at the end of each day's run, during which the vats were in operation from four to six hours, and not sterilized between batches. Pasteurization was performed at 143° F. for 45-minute periods. A further factor favorable to the development of the organism was the limited capacity of the separator which delayed the eventual cooling of the warm milk from the last vat. As Ayres and Johnson (1) have pointed out, the organism is readily killed by efficient steaming of the equipment, and for this reason equipment infections of this organism are not so likely as in the case of the spore-forming thermophilic bacteria.

THE SOURCE OF LACTOBACILLUS THERMOPHILUS

The organism was not isolated from the raw milk delivered to the plant. Six weekly platings from each of twenty-five shippers' milk were consistently free from the organism in the 0.01 cc. amounts tested. Attempts to stimulate growth by a prior four-hour incubation of the milk at 50° C. were not successful. More recently, four attempts to isolate the organism from the pasteurized milk and also from the raw milk from seven shippers to the Iowa State College pasteurizing plant, were unsuccessful. This evidence agrees with the statement of Ayres and Johnson, "It seems probable that the organism may be generally present in raw milk, but in small numbers, which may increase under suitable conditions. The source of the

organism is therefore not clear." These authors did isolate the organism from raw milk but their data show 10 shippers' milk free from thermophiles in the amounts tested, and that the high counts were due to a plant infection.

A similar organism, probably the same species, as judged by colony formation, morphology, and heat resistance, was recently the cause of trouble in another Oregon pasteurizing plant and it was traced to the milk from one shipper.

A close scrutiny of the bacteriological analyses of the milk supply of Corvallis for more than a year and of a much greater number of analyses of the Portland milk supply during the entire years 1926–27, has failed to reveal any outbreaks of thermophilic bacteria as shown by the Standard Plate Count, other than in the two cases mentioned. During this time, more than a hundred cases of high counts in pasteurized milk showing typical "pin-point" colonies have been investigated. In almost every case streptococci were the cause of the trouble. Studies on these organisms in regard to morphology, heat resistance, poor growth on standard nutrient agar and their saccharophilic nature have agreed with the results of Fay (2). It is a simple matter to differentiate the filamentous colony of *Lactobacillus thermophilus* from the more typical lens-shaped "pin-point" colonies characteristic of the thermo-tolerant streptococci.

As a by-product of this work it appears that under the conditions of the Standard Plate Count, spore-forming thermophiles have not been the cause of high-counts in the very large number of routine milk analyses observed. Although these organisms are wide-spread in nature they are not common in raw milk in large numbers as hundreds of platings on raw milk at 50° C. have revealed few and usually no thermophilic bacteria of any kind in the 0.01 cc. amount tested.

SIGNIFICANCE OF LACTOBACILLUS THERMOPHILUS

In the present investigation the organism appeared as a result of re-pasteurization, which is prohibited by most city milk ordinances. In the work of Ayres and Johnson, failure to sterilize the pasteurizing vat resulted in high counts of the organism. Hence, in these cases the presence of the organism has been indicative of faulty plant procedure. Cultures of the organism have been consumed without harmful results.

SUMMARY

An additional isolation of *Lactobacillus thermophilus* is reported. The organism agrees in almost every respect with the original description. Additional characteristics are recorded. The organism grows poorly on the nutrient agar used in routine milk platings, and the indistinct filamentous

colony may be "pin-point" in size. The colony can easily be distinguished from the lens-shaped "pin-point" colonies of the thermoduric streptococci. Much better growth is obtained when Bacto-Proteose peptone is substituted for ordinary peptone.

As in the original isolation *Lactobacillus thermophilus* is found to be the causative organism of high bacterial counts in a pasteurized milk supply. It would appear that the organism is not of common occurrence in milk. The particular growth requirements and the relatively short period of viability characteristic of the organism may also explain why it has not been isolated more frequently.

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SOME OBSERVATIONS ON THE EFFECT OF TEMPERATURE OF PASTEURIZATION ON THE ACID COAGULATION OF SKIMMILK*

L. M. THURSTON AND J. L. BARNHART
Department of Dairy Husbandry, West Virginia University
Morgantown, W. Va.

In studying the effect of temperature of pasteurization on the manufacture of cottage cheese, it was noticed that coagulation by culture-developed lactic acid occurred noticeably sooner when the skimmilk had been pasteurized for 30 minutes at 85°C. (185°F.) than for the same period at 62.8°C. (145°F.). This observation led to some conjecture as to the relation of temperature of pasteurization to the phenomenon of acid coagulation. An experiment was therefore planned to determine the effect of temperature of pasteurization of skimmilk on the time required for coagulation and on the curd tension, total acid developed, and H-ion concentration, each at the time of coagulation.

For this study mixed herd milk was separated and the skimmilk divided into six 600 cc. portions. Five of these were pasteurized in liter pyrex beakers at 62.8°C. (145°F.), 68.3°C. (155°F.), 73.9°C. (165°F.), 79.4°C. (175°F.), and 85°C. (185°F), respectively, each for a holding period of 30 minutes. Pasteurization was accomplished with a water bath heated by a gas flame. Each beaker was constantly watched during the heating and holding periods so that the holding temperature was controlled within one degree of that stated. The milks were cooled as soon as the holding period ended and were stored over night in an ice-box. The sixth sample was not heated. On the following morning duplicate samples of 200 grams from each were weighed into 250 cc. pyrex beakers, eight grams of active lactic culture added, all samples warmed to 30°C. in a water bath, then set in a constant-temperature oven at 30°C. A Hill[1] curd knife was placed in each beaker. In every case coagulation was allowed to progress until a jelly-like curd was evident. The curd tension was then determined by attaching a sensitive spring balance to the curd knife and drawing it slowly through the curd. The curd tensions recorded represent in each case the total pull in grams less the weight of the knife. Acidity was determined by titration with tenth-normal sodium hydroxide to a faint

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[1] HILL, R. L. The physical curd character of milk and its relationship to the digestibility and food value of milk for infants. Utah Agr. Exp. Sta. Bul. 207. 1928.

pink with phenolphthalein, and calculated as per cent lactic acid. Hydrogen-ion concentration was determined by means of a Leeds and Northrup laboratory potentiometer using a hydrogen electrode. Five complete trials were conducted.

Effect on curd tension—The effects of the various temperatures on the curd tension at coagulation are shown in figure 1. This curve was drawn

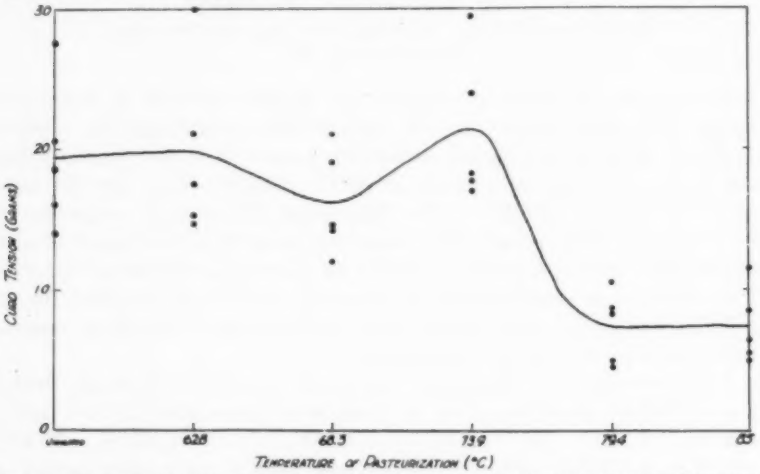

FIG. 1. RELATION OF TEMPERATURE OF PASTEURIZATION TO CURD TENSION OF SKIMMILK AFTER CURDLING.

free-hand through the arithmetic means of the tensions found for each temperature. The scatter diagram of the five trials is also shown.

The significant effect shown here is that curd tension of the acid coagulum is lower for skimmilks pasteurized at 79.4° and 85°C. than for those pasteurized at the lower temperatures or not heated. Variations in the curve at the lower temperatures used probably are not significant. Hill[1] found that duplicate determinations made by the use of his knives and balance sometimes varied as much as 10 grams, and in two cases such variations occurred in this work. It is likely that the variations in these data were due to experimental errors resulting from the use of a method not adaptable to precise determinations.

The fact that high-temperature pasteurization causes, on acid coagulation, a softer curd than results from the use of lower temperatures of pasteurization has often been observed. This effect has particular application in the manufacture of culture buttermilk and cottage cheese. The acid coagulum from skimmilk pasteurized at 73.9°C. or higher for a thirty-minute holding period appears to consist of smaller particles than the

coagulum from skimmilks pasteurized at lower temperatures or not heated, and the curd breaks and shatters more readily on stirring. In the manufacture of culture buttermilk this property is desirable but in cottage cheese making it is undesirable, being responsible for the breaking of curd flakes on stirring during the "cooking" process.

Effect on H-ion concentration and total acidity—The relationship of temperature of pasteurization to H-ion concentration and total acidity are shown in figure 2. Total acidity at coagulation was higher for skimmilk

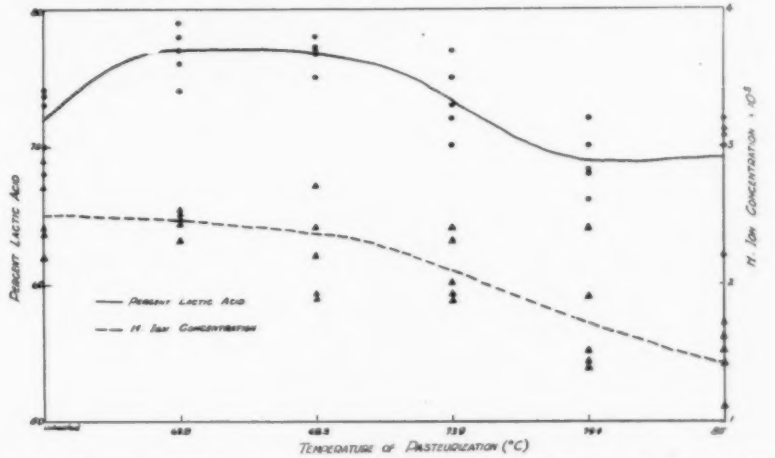

FIG. 2. RELATION OF TEMPERATURE OF PASTEURIZATION TO ACIDITY AND H-ION CONCENTRATION AFTER CURDLING.

pasteurized at 62.8°C. than for unheated skimmilk, but showed a gradual and uniform decrease as the temerature of pasteurization was increased. H-ion concentration at coagulation was the same for the unheated skimmilk and for that pasteurized at 62.8°C., but showed a gradual decrease as the higher pasteurization temperatures were used. One additional sample was heated to 92°C. and held for 30 minutes. When coagulated by the method used for the other samples its total acidity was 0.65 per cent and its H-ion concentration 0.93×10^{-5}, both of which were lower than any of the results at 85°C. The pH values are shown in the accompanying table.

There is evidence here that heat acts upon casein so that its coagulation may be brought about with less acid than is necessary in unheated milk. Whittier and Benton[2] have shown that continued boiling of whole milk causes a gradual increase in acidity and H-ion concentration. In their studies coagulation occurred at a pH of 5.6. The fact that coagulation

[2] WHITTIER, E. O. and BENTON, ANNE G. The formation of acid in milk by heating. JOUR. DAIRY SCI. 10: 126. 1927.

occurred at pH 5.6 when the temperature was at boiling is not surprising, as it is generally understood that heating milk of relatively low acid content brings about coagulation; but data herein presented show that previous heating followed by coagulation at a relatively low temperature produced the same apparent effect, though somewhat smaller in magnitude. These facts indicate that heat applied to milk produces two effects; *i.e.*, it produces acid (2), and it affects the casein so that acid precipitation occurs at a H-ion concentration lower than the iso-electric point of normal unheated milk by an amount that is roughly proportional to the intensity of the heat treatment.

The relation of temperature of pasteurization to pH at coagulation

TEMPERATURE TREATMENT	pH (AVE. OF FIVE TRIALS)
°C.	
Unheated	4.598
62.8	4.607
68.3	4.611
73.9	4.673
79.4	4.769
85	4.839
*92	*5.027

* Single trial.

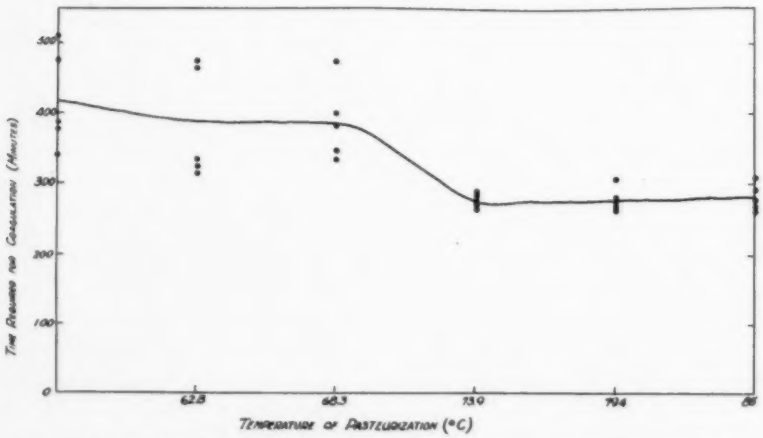

FIG. 3. RELATION OF TEMPERATURE OF PASTEURIZATION TO TIME REQUIRED FOR CURDLING.

Effect on time required for coagulation—The effect of the various temperatures on the time required for coagulation is shown by figure 3. The curve was drawn free-hand through the arithmetic averages of the time periods required for coagulation at each temperature. A rather sharp reduction in the time required for coagulation occurred between the skim milks pasteurized at 68.3° and those pasteurized at 73.9°C. Little change occurred between the times required for the group including the unheated skim milks and those pasteurized at 62.8° and 68.3°C., while still less change occurred in the group including the skim milk pasteurized at 73.9°, 79.4°, and 85°C., respectively.

This effect is explained, in part, by the fact that high-temperature pasteurization makes it possible to precipitate casein with less acid than is needed for unheated skimmilk, or skimmilk pasteurized at lower temperatures, as already pointed out. However, the differences in amount of acid required as the higher temperatures were used were relatively small and their change from low to high-temperature treatment produced a uniformly sloping curve not comparable to the curve of the time periods required for coagulation due to the sharp change of the latter between the temperature at 68.3° and 73.9°C. None of the data from these trials explain this irregularity of the time curve.

The time factor in acid coagulation has some significance in the manufacture of culture buttermilk and cottage cheese. The coagulation time is markedly reduced by increases in the intensity of previous heat treatment.

SUMMARY

Increasing the temperature of pasteurization of skimmilk was found to have several significant effects on its coagulation by lactic culture. The firmness of the coagulum, or the curd tension, at the time a jelly-like curd first formed, was roughly twice as great for raw skimmilk and skimmilks pasteurized at 62.8°, 68.3°, 73.9°C. as for those pasteurized at 79.4° and 85°C.

Increase in temperature of pasteurization reduced the H-ion concentration and also the amount of acid present at the time of coagulation. This decrease was slight up to 68.3°, above which the change was more marked with increasing temperature.

The time required for coagulation under uniform setting conditions was little changed by pasteurization at temperatures up to 68.3°, but was reduced materially at 73.9°C., beyond which there was little further change.

INFLUENCE OF ARSENICAL DIPPING ON YIELD OF MILK BY DAIRY COWS

P. T. DIX ARNOLD, W. M. NEAL AND R. B. BECKER

Department of Animal Husbandry, Florida Agricultural Experiment Station, Gainesville, Florida

INTRODUCTION

Since the advent of tick eradication through cooperation of federal and state authorities in eradicating the cattle fever tick, *Margaropus annulatus*, the immediate effect upon milk flow has been questioned. An appreciation of the insignificant effect of the arsenical dipping on milk yield should stimulate efforts towards complete eradication of this tick in the remainder of this, and other countries. For interpretation of experimental work in progress at the Florida Agricultural Experiment Station, it was necessary to measure the magnitude of the effect upon milk production. The dairy cows in the station herd were only very slightly infested with fever ticks, and hence no measure of comparison is intended in this paper between tick-infested and tick-free conditions.

REVIEW OF LITERATURE

Three investigations of the effects of the use of arsenical solutions, as employed in tick eradication, have been reported. Each of these was conducted at about the same time with selected groups of cows in single lactation periods. Scott,[1] at the Florida station, dipped nine cows for six consecutive times (54 cow-dips) at 14-day intervals. Milk records of five days preceding and succeeding the dipping were compared, and a total decrease of 8.68 per cent in milk yield noted.

McClain[2] reported 10 cows dipped seven times, or 70 cow-dips, in South Carolina with an average decline of 10.6 per cent in milk production in the two-day period after dipping. The first four dips caused a decline of 14.8 per cent milk and 8.9 per cent in butterfat production. The last three dips caused a loss of only 1.9 per cent in milk yield and an increase of 10.6 per cent in butterfat yield. The last three dips are hardly significant, since the decrease in milk yield was approximately the same as expected from advance in lactation.

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[1] Scott, J. M. How dipping affected the milk flow. Fla. Agr. Exp. Sta. Ann. Rept. 1915: xx–xxi.

[2] McClain, J. H. Eradication of the cattle tick necessary for profitable dairying. U. S. D. A. F. Bul. 639: 1–4. 1914.

A question arose as to whether the decrease in production was caused by the shock of dipping, or by arsenical absorption. To determine this point, Woodward, Turner and Curtice[3] obtained records from 20 cows which were sprayed rather than dipped at four consecutive bi-weekly intervals. By comparing milk yields for three preceding days with those of the five days after spraying, they noted an average reduction of 6.1 per cent in production. The cows required from three to five days to recover from the effect of the spray.

METHODS OF INVESTIGATION

The systematic tick eradication campaign in Alachua county was conducted by Federal and State authorities in accordance with the Florida Laws, Rules and Regulations governing tick eradication. The standard arsenical solution was used which contained from 0.18 to 0.20 per cent arsenious oxide (As_2O_3). All cattle in the county were dipped at 14-day intervals from March, 1930, to May, 1931, inclusive, with the exception of the months of January, February and March, 1931, when a 28-day interval was allowed. A total of 29 dip days was entailed with the Experiment Station herd during the campaign in this area. However, even before the campaign, ticks rarely could be found on any of the cattle in the herd.

The data presented herein were obtained from the milking cows in the Experiment Station dairy herd. These cows were driven one-half mile down a lane to the vat, and dipped shortly after the morning milking. They were turned on pasture during the day.

Within the province of this paper, a day's milk constitutes the total milk obtained at the afternoon and following morning milkings. The cows were milked at 5:00 A. M. and 3:00 P. M. daily, except on Sundays when the afternoon milking started at 2:30 P. M. with all but the cows on feeding experiment. Every milking of the cows in the station herd was used in the tabulations.

Since Alachua County, Florida, is located in the southern portion of the temperate zone close to large bodies of water, the climatic conditions differ from those of other regions. Winter is here defined as the interval between the average date of first killing frost in autumn and the last frost in spring. Tabulations of mean temperatures and of average rainfall by months (from Florida Agr. Exp. Sta. Bul. 200) were used to delineate "summer,"—the months of June to September inclusive. Spring and autumn were thus automatically defined. In addition to average climatological data upon which seasons are here defined, the actual rainfall and mean temperatures experienced during the eradication campaign, are shown in table 1.

[3] Woodward, T. E., W. F. Turner and C. Curtice. Effect of the cattle tick upon milk production of dairy cows. U. S. D. A. Bul. 147: 14–16. 1915.

TABLE 1

The 17-year average and the 1930–1931 actual climatic conditions as related to seasons in Alachua County, Florida

MONTH	AVERAGE DATES OF KILLING FROSTS		MEAN TEMPERATURES				RAINFALL		SEASONS
			MINIMUM		MAXIMUM				
	Average	Actual	Average	Actual	Average	Actual	Average	Actual	
			degrees	*degrees*	*degrees*	*degrees*	*inches*	*inches*	
March (1930)	2nd*	5th	51.9	48.5	75.5	67.8	3.21	6.49	Spring
April			57.3	57.7	81.3	80.2	2.21	2.12	"
May			63.3	63.4	86.6	89.2	3.11	4.99	"
June			68.8	68.3	89.7	85.9	6.57	12.03	Summer
July			70.7	71.6	90.1	92.4	7.32	3.10	"
August			70.9	69.0	90.7	90.9	6.76	3.40	"
September			69.0	70.9	88.9	90.7	5.65	8.64	"
October			62.0	57.5	82.7	80.1	2.84	1.08	Autumn
November	27**	29th	50.9	51.2	74.0	72.4	2.04	1.68	"
December			47.4	42.2	68.9	60.5	3.22	4.90	Winter
January (1931)			47.1	42.4	68.5	65.1	3.31	4.14	"
February			47.0	45.1	69.8	67.5	2.87	2.72	"
March	2nd*	5th	51.9	46.7	75.5	69.5	3.31	5.05	Spring
April			57.3	55.0	81.3	78.4	2.21	2.83	"
May			63.3	61.1	86.6	85.4	3.11	6.11	"

* Last killing frost in spring.
** First killing frost in autumn.

PRESENTATION OF DATA

The seasonal distribution of dippings, when the seasons were defined as outlined in table 1, was: spring, 7; summer, 13; autumn, 4, and winter, 5. Milk yields for the entire cow herd during the tick eradication campaign have been tabulated by seasons. Average daily milk yields, weighted for the entire 826 cow-dips, were calculated. With cows freshening and going dry in approximately equal numbers thruout the period, it was theoretically possible to neglect the decline in production due to the advance in lactation period as a factor affecting daily milk yield.

The decline in milk production for each day, and for the week after dipping for the different seasons and the entire tick eradication period, is presented in tables 2 and 3.

TABLE 2

Average daily milk yield per cow, by seasons, during the tick eradication campaign

"DAYS"*	SEASON AND NUMBER OF DIPPINGS				WEIGHTED AVERAGE
	Spring 7	Summer 13	Autumn 4	Winter 5	
	pounds	*pounds*	*pounds*	*pounds*	*pounds*
Before dipping					
7	15.88	17.38	16.60	13.84	16.29
6	15.88	17.15	16.97	13.82	16.26
5	15.93	17.47	16.89	14.10	16.44
4	15.90	17.67	16.67	13.88	16.46
3	15.75	17.40	17.00	13.86	16.44
2	16.23	17.52	16.54	13.36	16.29
1**	16.48	17.30	16.70	13.60	15.88
After dipping					
1	14.74	16.25	15.99	12.82	15.28
2	16.03	16.67	16.02	13.18	15.67
3	16.34	17.08	16.57	13.28	16.04
4	16.17	17.14	16.84	13.50	16.27
5	15.98	17.28	16.32	13.42	16.24
6	16.00	17.32	16.57	13.62	16.29
7**	16.34	16.95	15.79	13.44	16.06

* A "day" constitutes the P. M. and following A. M. milkings.

** Sunday afternoon milking was one-half hour earlier, except with cows on feeding experiment.

DISCUSSION OF RESULTS

With these 826 cow-dips through fifteen months of dipping under state and federal regulations and Florida climatic conditions, it has been pos-

TABLE 3

Percentage milk yield on seven days following dipping, using the average of the preceding seven days as 100 per cent

"DAY"	SEASON				WEIGHTED AVERAGE
	Spring	Summer	Autumn	Winter	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
1	92.08	93.32	95.37	93.03	93.78
2	100.14	95.73	95.54	95.65	96.17
3	102.08	98.09	98.82	96.37	98.44
4	101.02	98.43	100.43	97.97	99.85
5	99.83	99.24	97.33	97.39	99.67
6	99.96	99.47	98.82	98.84	99.97
7	102.08	97.34	94.17	97.53	98.56
Average	99.58	97.37	97.21	96.68	98.06

sible to verify earlier work and to show a decline in milk production lasting from three to four days after dipping. However, the percentage loss observed under these conditions was considerably lower (1.94 per cent, comparing previous and succeeding weeks' milk yields) than given in earlier reports. Since previous studies were all made with cows in single lactation periods, the decline in production given would include the normal decline due to the advance in lactation as well as that due to dipping. In this study, cows freshened and went dry in approximately equal numbers during the entire period, which would theoretically eliminate the effects of advance in lactation, so that the declines observed can be attributed almost completely to the effects of the dipping.

Apparent recovery was most rapid in the spring, followed in turn by autumn, summer, and winter. More rapid recovery of milk flow during the spring months may possibly be due to the stimulating influence of early pasture. Slower recovery, requiring four to five days, in the winter is associated with the shock of dipping and the chilling of the animal, together with a lack of green pasture. Declines for the first four days after dipping for the entire period, were 6.22, 3.83, 1.56 and 0.15 per cent respectively. No tolerance to arsenic absorption was developed, as decline in milk production occurred after each dipping during the whole eradication campaign.

The largest decline in milk yield was noted on the second milking following the dip, and appeared to be caused by slow absorption of arsenic through the skin, rather than by the immediate shock and excitement of dipping. This is confirmed by the loss of milk observed by Woodward and associates, who sprayed the cows with arsenical solution, rather than

dipped them. They noted a 6.1 per cent decrease in milk yield and a three to five day period of recovery. That the chilling and excitement of dipping are small factors, except in cold weather, is indicated from this work.

SUMMARY AND CONCLUSIONS

A total shrinkage in milk flow of only 1.94 per cent, comparing succeeding with previous week's milk yield, was observed in a tabulation of 826 cow-dips at the Florida station during the tick eradication campaign in Alachua county. The greatest decrease in production was in the second milking after the cows were dipped, indicating that the effect was produced by arsenic absorption rather than by the excitement and shock of dipping. No tolerance to the arsenic was developed. The effect of dipping was greatest in the winter, followed by autumn, summer, and spring, indicating that low temperatures emphasize the effect of dipping. Shrinkage in milk yield was 6.22, 3.83, 1.56 and 0.15 per cent consecutively for the first four days after dipping. The decline in average daily milk yield due to advance in lactation, was practically eliminated in these tabulations by the fact that cows freshened and were turned dry in approximately equal numbers throughout the campaign.

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THE VIABILITY OF LACTOBACILLUS ACIDOPHILUS AS AFFECTED BY FREEZING IN A SHERBET MIXTURE*

CHAS. C. PROUTY AND H. A. BENDIXEN

Washington Agricultural Experiment Station, Pullman, Washington

Lactobacillus acidophilus milk is unpalatable to many persons. Some object to the cooked flavor brought about by the high pasteurization temperature used in its preparation, while others dislike the flavor imparted during the process of fermentation. Partially to overcome these objections and partially for exploitation purposes, numerous attempts have been made to market the organism in the form of tablets, powders, semi-solid candy-coated preparations and broth cultures. Numerous investigators have shown the tablets, powders and candy-coated preparations to be of little value and usually worthless as conveyors of viable organisms. In many instances, broth cultures have been found to be devoid of or extremely low in number of viable organisms by the time they reached the consumer. For instance, Black (1) made a quantitative analysis of a broth culture prepared for distribution by a commercial laboratory. This preparation was labeled to contain 200,000,000 viable organisms at the time of manufacture and 100,000,000 viable organisms at the expiration date one month later. He found a maximum plate count of 48,000,000 per cc. three days after the date of manufacture. Unpublished observations made by us have resulted in similar findings. Of the preparations employed as conveyors of *L. acidophilus* organisms, milk cultures have shown the highest average bacterial counts. All investigators, however, do not agree as to the most desirable method of maintaining the viability of these organisms in milk cultures.

REVIEW OF LITERATURE

Low temperatures improve the palatability of *L. acidophilus* milk. However, it has been the prevailing opinion among bacteriologists that the number of viable *L. acidophilus* organisms in milk is reduced more rapidly when the product is stored at refrigerator temperature than when stored at room temperature. Kopeloff (2), as well as Reichart and Davis (3), have presented data to uphold this belief. Trammal and Hastings (4), found the organisms to remain viable for a longer period of time at 10°C. to 13°C. than at 22°C. Black (1) working with a number of strains of both *L. acid-*

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ophilus and *L. bulgaricus* found a lower reduction in numbers at refrigerator temperature, 9°C. or even 0°C., than at 37°C. He noted little difference in the viability of *L. acidophilus* and *L. bulgaricus* in milk kept for several days at refrigerator and room temperatures. Black and Harris (5) found a storage temperature of 9°C. to result in a smaller reduction of viable organisms than storage at room temperature. They found the largest number of organisms present at low acidities and the greatest destruction of organisms in the most acid samples. From these results, they concluded that the reaction of the stored culture is a more important factor in decreasing the number of viable organisms than the storage temperature.

In a recent study, Kulp (6) found the continued life of *L. acidophilus* to be dependent upon two factors; namely: the initial content of acid or some related metabolic substance and the temperature of storage. He found that commercial *L. acidophilus* milk of good quality and containing a satisfactory number of organisms which would remain viable for two days to one week, might be stored at 5°C., provided the initial acidity did not exceed 0.65 per cent. To insure viability for periods longer than one week, he found it necessary to reduce contamination to a minimum, to keep down acidity and to maintain the storage temperature between 12°C. and 16°C., the temperature for optimum viability being 16°C. with an initial acidity of 0.65 per cent. Myers (7) recently developed a sweet milk product carrying large numbers of viable *L. acidophilus* organisms to which he gave the name unfermented *L. acidophilus* milk. The *L. acidophilus* organisms are grown in a sterilized medium and freed from it without contamination. They are then suspended in pasteurized milk in numbers comparable to those found in a good cultured acidophilus milk. He found the death rate of *L. acidophilus* in such a preparation to be very low when stored at 2°C. to 5°C. for a period of four to five days and attributed this low death rate to the low acid content of the product.

EXPERIMENTAL

To increase the palatability of *L. acidophilus* milk, it was sweetened, flavored, and frozen into a sherbet. In addition to a high sugar concentration, the organisms were exposed to extremely low temperatures while the product was in storage. Since the therapeutic benefit derived from any *L. acidophilus* product is dependent upon the numbers of viable organisms at the time of consumption, a study was made of the viable organisms remaining in the sherbet at intervals throughout a seven-day storage period.

Each culture of *L. acidophilus* used in this study was transferred daily for a period of several days prior to use. From this actively growing culture a transfer was made into a volume of sterile skim milk sufficient to prepare several sherbet mixes. After incubation at 37°C., usually for

a period of twenty-four hours, the necessary ingredients were added and the mixture frozen into a sherbet. The general composition of the mixes was as follows: sugar 32 per cent, *L. acidophilus* milk 48 to 68 per cent, flavoring material 0 to 20 per cent. In a few instances, some variation from the above was made in the sugar content. The flavoring materials used were fresh lemon and orange juices, crushed pineapple, citric acid, lactic acid, grape juice, and artificial walnut and coffee extracts. The freezing process was carried out in a two-quart freezer which previously had been sterilized in the autoclave. Plate counts using Bacto-dehydrated galactose whey agar were made of the cultured milk, the freshly prepared mix, immediately after freezing and after storage periods of 1, 2, 3, 5 and 7 days. Acid determinations were made of both the cultured milk and the prepared mix. The storage temperatures varied from –17°C. to –25°C. A total of 28 sherbets was studied. The results are presented in table 1.

DISCUSSION

The results presented in table 1 show a variation in the resistance of the strains of *L. acidophilus* to the conditions under which they were subjected when frozen in a sherbet mixture. Strain A showed the most rapid reduction in numbers of viable organisms. After five days of storage at temperatures below –17°C. the viable organisms in sherbets carrying this strain were reduced to only a few million per cc., while under similar conditions several hundred millions of viable organisms per cc. were present in the sherbets carrying the other strains of *L. acidophilus*. Pronounced reductions of organisms of strain A were evident after one day of storage. There were fewer initial organisms in cultures of this strain than in cultures of other strains. Since all of the cultures were incubated under similar conditions prior to being frozen into sherbets, it is apparent that the low initial numbers of organisms of strain A and their inability to remain viable during storage were due to inherent characteristics of the strain.

A variation in viability was found to occur in cultures of the same strain. This variation was very pronounced in cultures of strain O represented by sherbets 12 and 14. Sherbet 12 having an acid content of 0.75 per cent and an initial count of 1,670 million organisms per cc. contained 160 million viable organisms per cc. after a storage period of seven days, while sherbet 14 with an initial acid content of 0.65 per cent and an initial count of 900 million organisms per cc. contained 700 million viable organisms per cc. after a similar storage period. Significant variations occurred in cultures represented by sherbets 2 and 8, 12 and 15, 20 and 23. A study of these sherbets, in which two different cultures of the same strain are represented, showed no correlation between either the initial acidity of the culture or the acidity of the mix and the numbers of viable organisms re-

TABLE 1

Viability of Lactobacillus Acidophilus in Sherbets

NO. OF SHERBET	STRAIN	CULTURE	FLAVOR	PER CENT SUGAR	PER CENT ACID		BACTERIAL COUNT IN MILLIONS PER CC.							
										Time in Storage				
					Culture	Mix	Culture	Mix	After freezing	1 day	2 days	3 days	5 days	7 days
1	K	1	None	32	0.57	0.47	1010	650	640	840	735	750	700	—
2	K	1	Lactic acid	32	0.57	1.62	1010	580	—	750	545	550	500	—
3	K	2	Lactic acid	32	0.40	1.90	830	640	400	360	315	290	240	—
4	K	2	None	32	0.40	0.36	830	700	690	600	510	500	480	—
5	K	3	None	35	1.15	0.88	3040	2300	1720	1530	—	1550	—	1400
6	K	3	None	20	1.15	0.95	3040	2080	1880	1800	—	1860	—	1600
7	K	4	Pineapple-lemon	18	1.02	1.17	1670	1470	1300	1100	1145	1100	1000	930
8	K	4	Orange-lemon	32	0.73	1.45	1500	720	680	670	624	533	488	425
9	O	1	None	32	0.64	0.51	1107	1010	—	755	700	508	240	115
10	O	1	Lactic acid	32	0.64	1.97	1107	780	—	350	240	139	—	3
11	O	2	Lemon	32	0.90	1.95	2528	1810	—	900	—	440	48	26
12	O	2	None	32	0.90	0.75	2528	1670	—	1400	—	1050	347	160
13	O	3	Lemon-orange	32	0.57	1.95	1080	720	470	280	260	150	30	8.4
14	O	4	None	32	0.85	0.65	1300	900	1000	890	833	870	—	700
15	O	4	Lactic acid	32	0.85	1.52	1300	840	660	560	550	350	—	250
16	H	1	Lemon-orange	32	0.75	2.05	1550	540	460	—	—	450	240	—
17	H	1	None	32	0.75	0.63	1550	1000	730	—	—	820	555	—
18	H	1	None	20	0.75	0.63	1550	1250	750	—	—	790	600	—
19	A	1	None	32	0.48	0.40	412	340	—	300	—	98	53.5	—
20	A	1	Citric acid	32	0.48	1.08	412	360	—	210	—	27	5.0	—
21	A	1	Citric acid	32	0.48	1.37	412	320	—	180	—	25	5.0	—
22	A	2	None	32	1.10	0.78	850	620	600	—	210	180	—	160
23	A	2	Citric acid	32	1.10	2.10	850	680	490	—	180	160	—	20
24	A	2	Citric acid	32	1.10	3.40	850	560	512	—	42	35	—	3.5
25	A	3	Coffee	32	0.76	0.56	540	—	—	52	36	—	—	11
26	A	4	Coffee	32	0.83	0.61	410	—	—	36	27	—	7	—
27	A	5	Walnut	32	0.82	0.74	386	—	—	92	69	—	98	—
28	A	6	Grape	32	0.53	—	310	—	—	75	73	—	—	18

Where there are no data counts were not made.

maining after a storage period of seven days. Whether or not these variations were due to the age of the cells or some combination of factors was not determined.

Another point of interest was the apparent relation of reaction to the death rate of the organisms. Several sherbets carrying organisms from the same lot of cultured milk were prepared in such a manner as to differ in reaction only. This was accomplished by the addition of fresh lemon and orange juices, citric acid, or lactic acid to one of the duplicate mixes. Where this was accomplished by the use of lemon and orange juices, these juices constituted from 10 to 20 per cent of the volume of the mix. This dilution resulted in a natural decrease in organisms per unit volume as compared with the mix not receiving such an addition. Where concentrated solution of citric or lactic acid were used the reduction in numbers of organisms per unit volume due to dilution was a negligible factor. Sherbets 1 and 2 were prepared from the same sample of cultured milk, the acid content of sherbet 2 being increased from 0.47 per cent to 1.62 per cent by the addition of lactic acid. After a storage period of five days, the viable organisms in these sherbets numbered 700 million and 500 million per cc. respectively. Similar comparisons were made with sherbets 3 and 4, 9 and 10, 11 and 12, 14 and 15, 16 and 17, 19, 20 and 21, and 22, 23 and 24, and in every instance a greater number of viable organisms were found in the sherbet having the smaller amount of titratable acidity. These findings are similar to those reported by Black and Harris (5) in which they found the greatest destruction of organisms during storage at refrigerator temperature to occur in the most acid samples of the series.

In sherbets 5 and 6, and 17 and 18, a comparison was made of the effect of sugar on the viability of the organisms. Sherbets 5 and 6 were prepared with sugar contents of 35 per cent and 20 per cent respectively, and sherbets 17 and 18 were prepared with sugar contents of 32 per cent and 20 per cent respectively. After seven days storage, sherbets 5 and 6 contained 1,400 million and 1,600 million viable organisms per cc. respectively and after five days storage sherbets 17 and 18 contained 555 million and 600 million viable organisms per cc. respectively. Taking into consideration the variation in dilution caused by the addition of the respective amounts of sugar the difference in counts in the sherbets used in this comparison was so small as to be of no significance.

A number of very palatable sherbets were prepared in which grape juice, artificial walnut and coffee flavoring materials were used. These materials, as well as lemon and orange juices and crushed pineapple, served in a highly desirable manner to mask the natural *L. acidophilus* milk flavor or the cooked flavor imparted during the pasteurization of the milk.

Whether or not therapeutic benefits would result from the use of these

sherbets prepared from *L. acidophilus* milk was not determined. According to Kopeloff (2) the average daily dose should be the equivalent of 1,000 cc. of a culture containing 200 million viable *L. acidophilus* organisms per cc. However, as reported by practicing physicians, the favorable results from the continued use of broth cultures prescribed in comparatively small daily doses along with lactose or mixtures of lactose and dextrose would tend to indicate that smaller doses are of benefit. It would seem that *L. acidophilus* sherbets of low acidity and carrying organisms of the more resistant strains if used within a period of seven days after manufacture, might possess therapeutic properties equal to if not better than the majority of broth cultures found on the market at the present time.

SUMMARY AND CONCLUSIONS

Twenty-eight sherbets were prepared from acidophilus milk and a study was made of the numbers of viable organisms remaining after 1, 2, 3, 5 and 7 days of storage at temperatures below –17°C. Four strains of *L. acidophilus* were used in the preparation of these sherbets.

A variation was found in the viability of these strains when exposed to the conditions prevailing in such a frozen product. The numbers of viable organisms in sherbets carrying strain A organisms were reduced to only a few million per cc. after five days storage, while under similar conditions several hundred millions of viable organisms were usually present in the sherbets carrying the other strains of *L. acidophilus*.

A variation in viability was found to occur in cultures of the same strain. Whether or not this variation was due to the age of the cells or some combination of factors was not determined.

A number of these sherbets were prepared so that they differed in reaction only. A more rapid reduction of organisms was found to occur in the sherbets of the higher titratable acidity.

The sugar concentration, as it was varied in the sherbet mixtures, caused no significant variation in the viability of the organisms.

It was possible to prepare sherbets in which large numbers of organisms remained viable after five to seven days of storage at temperatures below –17°C. when the more resistant strains of *L. acidophilus* with a high initial count were used and the titratable acidity was not permitted to become excessive, preferably not in excess of 1.0 per cent.

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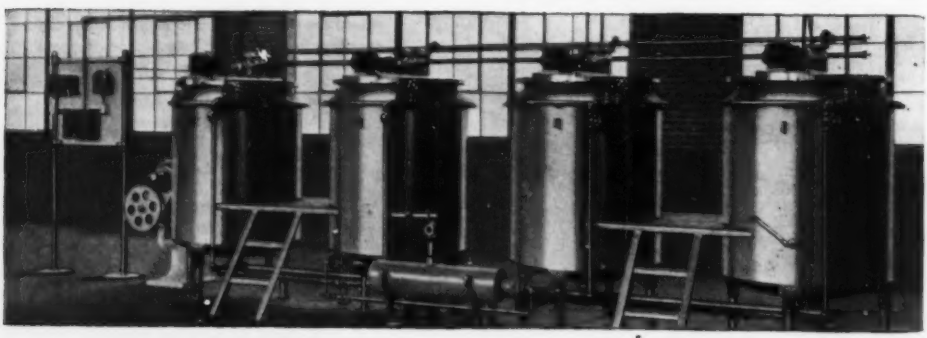

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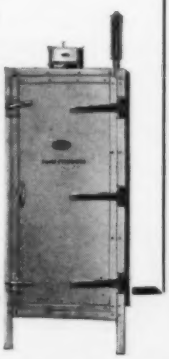

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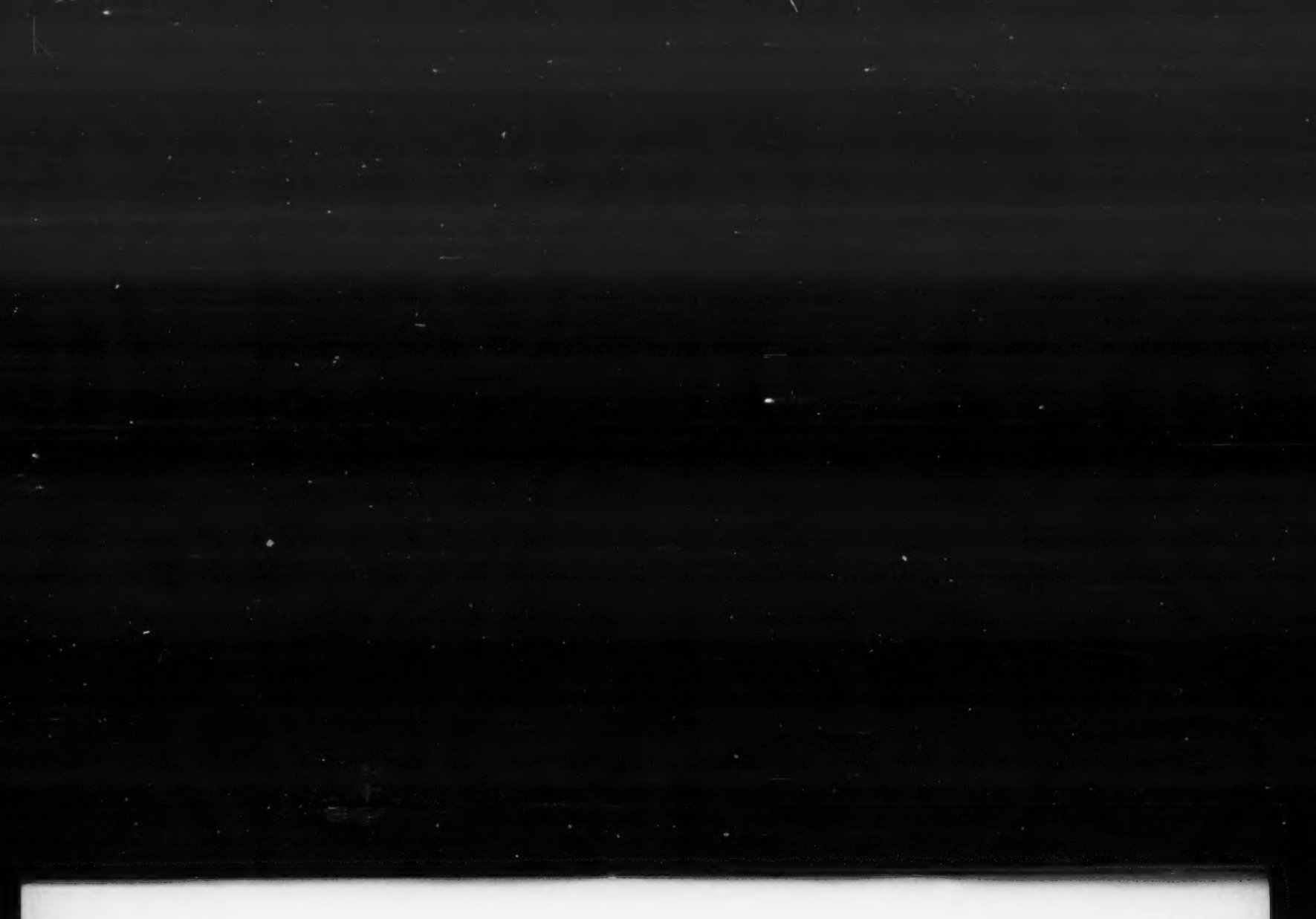

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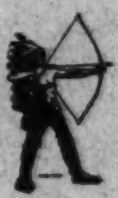

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